



IMPERIAL INSTITUTE  
OF  
AGRICULTURAL RESEARCH, PUSA.







**THE ANNALS OF  
APPLIED BIOLOGY**

LONDON  
CAMBRIDGE UNIVERSITY PRESS  
FETTER LANE, E.C. 4



CHICAGO: THE UNIVERSITY OF CHICAGO PRESS  
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# THE ANNALS OF APPLIED BIOLOGY

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AND

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VOLUME XIX

CAMBRIDGE

AT THE UNIVERSITY PRESS

1932

**PRINTED IN GREAT BRITAIN**

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THE FILTRATION OF THE VIRUS OF  
STREAK DISEASE OF MAIZE

BY H. H. STOREY, M.A., PH.D.

*(East African Agricultural Research Station, Amani.)*

(With 1 Text-figure.)

STREAK disease of maize, upon which a series of studies has been carried out (6, 7, 8), is in many respects a typical virus disease. Hitherto, however, the critical evidence which would place it certainly in the group of virus diseases—the filterability of its virus—has been lacking. Its inclusion in this group has rested upon the absence of a visible parasite and a general similarity in the clinical picture to that shown by diseases known to be caused by filterable viruses.

The reason for the lack of this piece of evidence has been the failure of all attempts to inoculate the streak virus into maize by artificial means. Consequently it was impossible to decide whether the virus was present in any given fluid; and the whole field of *in vitro* studies was closed.

A similar situation has been encountered in the investigation of many virus diseases. In the instance of curly-leaf disease of sugar beet, Carter (1, 2, 3) and Severin and Swezy (5) have overcome the difficulty by showing that leafhoppers may be successfully fed on nutrients through thin membranes; and that in this process they may take up the curly-leaf virus from the fluid and subsequently may be able to transmit it to healthy plants. By this means Severin and Swezy (5) demonstrated the filterability of the virus of curly leaf.

I have successfully applied this technique to the feeding of the vector of streak disease, *Cicadulina mbila* Naude, upon fluids containing the virus of streak disease. I have been able to show that the streak virus will pass filters of grades which are generally accepted as retaining visible organisms and in my experiments actually retained the control organism used. The virus, however, failed to pass certain grades of filters.

The proof of the filterability of a particular virus is of no considerable fundamental importance and I do not attempt to draw any fundamental conclusions from my results. I here record them briefly, since they fill an obvious gap in our knowledge of streak disease. They furthermore confirm the findings of Carter and Severin as to the practicability of their

## 2 *The Filtration of the Virus of Streak Disease of Maize*

technique for *in vitro* studies of viruses which are readily transmitted only by insects.

### TECHNIQUE.

*The virus fluid.* Young maize seedlings, infected with streak disease, were cut up and crushed, the roots and all leaves not bearing the chlorotic streak markings being rejected. From the crushed mass the juice was squeezed out through muslin by hand and clarified through paper pulp. To the clarified juice was then added an equal quantity of 20 per cent. sucrose solution. This mixed juice was the fluid used for filtration. To it immediately before filtration was added an emulsion of the control organism, *Bacillus pyocyaneus* (*Pseudomonas aeruginosa*)<sup>1</sup>.

*Filtration of the fluid.* In this work the following filters were used:

Chamberland candles, grades L1 and L3, 160 × 15 mm., with glazed ends.

Berkefeld candles, grades V and N, 60 × 17 mm., in glass mounts.

Seitz E. K. asbestos disc, 60 mm. diam., in the Uhlenhuth type of holder (i.e. the disc held by clamping screws)<sup>2</sup>.

All filters were set up for filtration under negative pressure, the fluid during filtration passing from the outside of the candles inwards. Before use all candles were washed by passing distilled water through under pressure. Bubbling tests were performed on all candles before being set up and again after the filtration had been performed. These tests revealed no gross defects. General bubbling occurred at about the pressures normal for the grades of filters used (4), pp. 78-9)<sup>3</sup>. The filters and all glassware used were sterilised in an autoclave for 20 min. at 125° C. Used candles were cleaned by boiling in a solution of sodium carbonate and by washing through with distilled water.

Immediately after filtration platinum loops of the filtrate were inoculated on to agar slopes.

The quinhydrone electrode apparatus was employed to determine the hydrogen-ion concentration of the fluids.

*Feeding of the fluid to leafhoppers.* I have used a feeding chamber, which is a slight modification of that used by Carter<sup>(1)</sup> (Fig. 1). The fluid is contained in a glass tube, the end of which is covered with a piece of "Baudruche capping skin"<sup>4</sup> (2) held in place by a ring cut from a rubber

<sup>1</sup> Supplied by the Medical Research Laboratory, Dar-es-Salaam.

<sup>2</sup> These discs were marked "Besonders feinporige Specialschichten 'Seitz E.K.'"

<sup>3</sup> Obtained from Paul Troeder, Belleville, New Jersey, U.S.A.

<sup>4</sup> All the available candles of the Berkefeld W grade showed general bubbling at pressures equal to or less than those shown by the N grade. They were therefore not used in this work.

bung, suitably bored. This bung fits the end of a glass tube, which forms the insect cage. The rubber bung and membrane form a flush surface closing the end of the tube completely and leaving no cracks into which hoppers can force their way. The apparatus is clamped in a sloping position so that the light from a window shows through the feeding membrane, to which the hoppers consequently move.

In my experiments I have usually allowed the leafhoppers to feed through the membrane for about 24 hours. The development of yeasts upon fluid exuded through the punctures in the membrane was apparently responsible for the heavy mortality among the hoppers if the period was considerably extended. After the period of feeding the hoppers were tested individually by the leaf-tube method(7) upon maize seedlings growing in an insect-proof greenhouse.

#### EXPERIMENTS.

In preliminary experiments no success was obtained in virus transmission when the leafhoppers were fed upon the leaf fluid alone, without added sugar. Additions of sucrose to concentrations of 5, 10 or 20 per cent. appeared to be about equally effective. With one of these concentrations and a 24-hour feeding period, usually about 50 per cent. of the hoppers became infective. Clarification of the fluid through filter paper or paper pulp did not render it less effective than the unclarified fluid.

A comparative experiment was carried out on the feeding of leafhoppers on the fluid after filtration through the five filters already mentioned. The

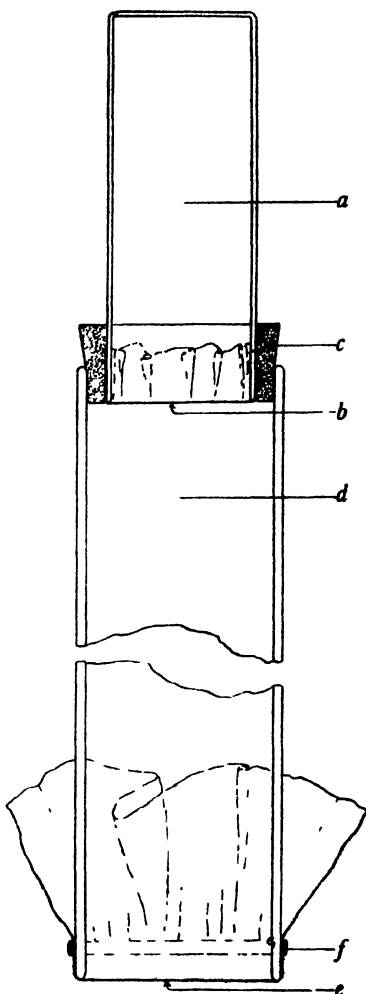


Fig. 1. The leafhopper feeding chamber, in section. The glass tube *a*, containing the feeding fluid, is closed by the membrane *b*, held in place by the rubber ring *c*. This ring fits the end of the glass tube *d*, 8 in. long  $\times$  1 in. diameter, the other end being closed by the muslin *e*, held in place by the rubber band *f*.

#### 4 The Filtration of the Virus of Streak Disease of Maize

relevant data concerning the filtration appear in Table I. It will be noted that the control organism, *Bacillus pyocyaneus*, passed both the "preparatory" grades of filters, Chamberland L1 and Berkefeld V. The remaining filters gave bacteriologically sterile filtrates. In Table II are presented the results of the feeding of hoppers upon the filtrates. They show that the virus passed the three filters, L1, V and N without any apparent loss. From the L3 filtrate, however, only a single hopper acquired the virus, while none acquired it from the E.K. filtrate.

Table I.

	Chamberland L 1	Chamberland L 2	Berkefeld V	Berkefeld N	Seitz E.K.
Previous use of filters	New	Once used	Once used	Once used	New disc
Bubbling tests (mm. of mercury)—before autoclaving	260	> 650	310	580	—
Bubbling tests (mm. of mercury)—after filtration	270	> 650	360	570	—
Filtration pressure (mm. of mercury)	400	400	400	400	400
Volume filtered (c.c.)	30	30	30	30	30
Approximate time of filtration (sec.)	90	150	45	150	180
pH—before filtration	5.8	5.8	5.8	5.8	5.8
pH—after filtration	5.9	6.1	6.1	6.4	6.1
Control organism—growth from filtrate	++	nil	+	nil	nil

Table II.

*Preliminary control test of leafhoppers. All hoppers, bred on a healthy plant, tested on three maize seedlings, which remained healthy.*

Nature of fluid fed to hoppers	Period of feeding hours	Test plants fed on by single hoppers		Control plants	
		Total	Infected	Total	Infected
Unfiltered clarified juice	20	15	6	24	nil
Chamberland L 1 filtrate	22.5	13	8	24	nil
L 3     "	23	17	1	23	nil
Berkefeld V       "	24	8	5	9	nil
N       "	24	15	7	20	nil
Seitz E.K.         "	22.5	17	nil	25	nil

In order to confirm the doubtful results obtained from the L3 and E.K. filtrates a second experiment was carried out. The filtration data in this experiment were as shown for the relative filters in Table I, except that the L3 candle had been twice previously used and the pH of the fluid was—before filtration, 5.9; after L3, 6.1; after E.K. 6.2. Again

only a small number of infections resulted from hoppers fed upon the L3 filtrate, while the E.K. filtrate again gave evidence of being virus-free (Table III).

Table III.

*All hoppers proved non-infective in preliminary test.*

Nature of fluid fed to hoppers	Period of feeding hours	Test plants fed on by single hoppers		Control plants	
		Total	Infected	Total	Infected
Unfiltered clarified fluid	24.5	19	13	20	nil
Chamberland L 3 filtrate	27	20	2	21	nil
Seitz E.K. filtrate	25	20	nil	20	nil

From these results it appears that the Berkefeld N is the most suitable filter for obtaining sterile filtrates containing the virus of streak disease.

#### SUMMARY.

Experiments have been carried out in the feeding of leafhoppers through membranes upon the juice of streak-diseased maize plants after passage through various filters. Tests of these leafhoppers have shown that at a pH of about 6 the virus passes through Chamberland L1, Berkefeld V and N filters, and less freely through Chamberland L3. The virus does not pass a Seitz E.K. filter disc.

I acknowledge the help received from Mr R. F. W. Nichols in the course of this work.

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(Received May 8th, 1931.)

## THE CHLOROTIC DISEASE OF THE HOP. II

BY PROF. E. S. SALMON AND W. M. WARE, M.Sc.

(*Mycological Department, South-Eastern Agricultural College, Wye, Kent.*)

IN our previous paper in this *Journal* (1930, xvii, 241) we described and figured a new virus disease of the hop to which we gave the name "Chlorotic Disease." The present paper records instances of the transmission of the disease by three methods.

### 1. GRAFTING.

#### A. *Healthy scions grafted on diseased stocks.*

(a) Plant Ref. No. V. 96. This chlorotic hop plant<sup>1</sup> was sent from Worcestershire in 1927 and planted out in that year in the Experimental Hop Garden at Wye. In 1929 it was grafted with two scions of the variety Fuggles; one died and the other remained healthy throughout 1929, reaching the height of 6 ft. and producing a few small cones. The plant was "dressed" (pruned) in November, 1929, but the base of the scion which had remained healthy was not cut away and the "hill" was earthed up (according to the usual practice) to a depth of 5 in. On May 28th, 1930, the scion (above the place of union with the stock) was producing four weak shoots, the longest of which was 10½ in., and the chlorotic disease was plainly visible in two of these. On August 4th two stems only were growing from the scion; one of these, 5 ft. high, was healthy; the other, 11 ft. high, bore chlorotic leaves, with characteristic distortion, to a height of 6 ft., but above that, healthy leaves. The laterals showed chlorosis on the basal pair of leaves. This was clearly, therefore, another case of transmission of the disease, although for one season's growth (1929) the scion had appeared healthy.

(b) Plant Ref. No. V. 97. This was of the same origin as V. 96. In 1929, of the two scions of Fuggles grafted, one remained healthy and the other showed chlorosis (see p. 245 of our previous paper). On November 13th, 1929, the scion which showed chlorosis was cut from the "hill" and planted in a pot. On May 8th, 1930, the stem of this scion after growth to a height of 38 in., with 13 nodes, showed chlorosis on four leaves (at the 6th and 7th nodes from the base), and the number of leaves

<sup>1</sup> Believed to be the variety Fuggles.

which showed chlorosis increased during the subsequent months. The scion which remained healthy in 1929 was left on the stock plant and earthed up. On May 28th, 1930, when examined, the scion was found to be dead, and the stock plant was producing only one shoot, 13 in. high, with 12 nodes. The leaves were small, chlorotic and misshapen, becoming smaller and smaller towards the apex of the shoot, until finally they were rudimentary or absent. The stipules were large and normal. Laterals, 3 to 4 in. long, were growing from the lower nodes, indicating a cessation of growth of the shoot. At the end of July, when next examined, this plant was found to be showing undoubted symptoms of mosaic disease. The six weak shoots present, 8 in. to 5 ft. high, all showed mosaic mottling of the foliage and no chlorotic symptoms. These six shoots all arose from one "strap" shoot remaining on the "crown" of the rootstock. The sudden appearance of mosaic disease on a plant formerly showing chlorosis is highly interesting. This plant (V. 97) was obtained direct from Worcestershire in 1927 as one of the variety Fuggles affected with chlorosis in the grower's hop-yard. The variety Fuggles, however, is not susceptible to the mosaic disease. A possible explanation of the present occurrence may be that the plant V. 97 was in reality some other variety, possibly the Mathon, a variety which is susceptible to the mosaic disease and, there is reason to believe, to the chlorotic disease also. The plant died during the winter of 1930-31.

(c) Plant Ref. No. AA .4. This chlorotic plant<sup>1</sup> was sent from Worcestershire in 1927 and planted out in the Experimental Hop Garden at Wye in 1928. In 1929 it was grafted with two scions of Fuggles; one was destroyed by accident, the other grew to a height of 6 ft. without showing any disease and bore a few normal hop cones. This scion was not removed from the "hill" when this was "dressed." During May, 1930, chlorosis was observed on the leaves of four bines growing from this scion, and during the season the symptoms shown were as pronounced as any that were visible on the four bines growing from the stock plant—and on these the most advanced symptoms of the disease were shown. Both sets of bine were vigorous and reached a height of 15 ft. It was noteworthy that the leaves (8 or 9 pairs) borne on the upper 4 feet of all the bines showed no chlorosis. On August 4th, 1930, the chlorotic bines from the scion of 1929 were in burr. This was a case, similar to that of V. 96, of the transmission of the disease, although the scion remained apparently healthy for the first year.

<sup>1</sup> Believed to be the variety Fuggles.



*B. Chlorotic scions grafted on healthy plants.*

As mentioned in our previous paper (p. 246), four healthy pot-plants of the variety Fuggles<sup>1</sup> were grafted in 1929 with scions taken from chlorotic plants<sup>2</sup>. The scions, with one exception which died after two months, grew and persisted throughout the season. They reached heights varying from 7 in. to 4½ ft., and though some of the lower leaves showed evident chlorosis, the disease did not become apparent in the later-produced leaves higher up the stems. In 1929 there was no visible transmission of the disease from scion to stock. In 1930<sup>3</sup>, clear evidence of such transmission appeared, details of which are given below.

Plant 1 (§ 24), 1929. On May 3rd, five bines were present; two (4½ ft. and 3 ft. high) were cut down nearly to ground level to provide stocks for the insertion of two scions from the chlorotic plant V. 96. These scions bore only two pairs of leaves showing slight deformity and yellowing<sup>4</sup>; during the season they reached heights of 4 and 4½ ft. The three bines of the stock plant all grew to 10 ft. in height and remained healthy.—1930. On April 20th, the first chlorotic symptoms were visible on the stock plant, and detailed notes were made one week later. Four bines (5½–7 ft., with 12–15 nodes) were present; all bore healthy leaves at the lowest 5–7 nodes, but above this (*i.e.* at 25–34 in. from the ground) to the apical bud, all the leaves on all four bines showed the symptoms of chlorosis.

Plant 2 (§ 23), 1929. On May 3rd, two bines, 3 ft. high, and four runner shoots, 1 ft. high, were present. The two bines were cut down to provide stocks for two scions taken from the chlorotic plant AA. 5. These scions were very young and were only 4 in. long; one showed deformity of leaf outline and yellow colour and the other no distinct symptoms of chlorosis. By July 16th, both had grown to a height of 7 in., but they appeared unhealthy and showed no promise of further growth. On July 30th, one scion was dead; the other, with all its leaves

<sup>1</sup> The plants of Fuggles used were obtained some years ago from a grower in Worcestershire, and came from three different hop-yards, A, B, and C. Of the six plants used as stocks, one (§ 21) came from C, one (§ 22) from A, and two (§§ 23, 24) from B. All were kindly supplied to us from the Research Station, East Malling.

<sup>2</sup> These plants (V. 96, AA. 4, AA. 5) were received during 1927 from a hop-grower farming near Tenbury, Worcestershire, and were later planted in the Experimental Hop Garden, Wye.

<sup>3</sup> The four plants were kept in an unheated glasshouse during the winter and were not placed out-of-doors until May 7th, 1930.

<sup>4</sup> Owing to damage by flea beetles, it was impossible to make detailed notes of the symptoms shown.

fallen, still showed some green colour on the stem. Two bines (8 ft. high) on the stock plant remained healthy.—1930. On April 12th, the first symptoms became apparent. The stock plant bore only one vigorous bine, 6 ft. 8 in. high, with 14 nodes, and the leaves at the 9th and 10th nodes showed chlorosis. At the nodes above this (11th–14th) the leaves were hardly free from the protecting stipules and the presence of the disease could not be confirmed. Sixteen days later, when the bine was 10 ft. high, with 19 nodes, it showed typical symptoms of chlorosis on all the leaves from the 9th node upwards, and the disease remained throughout the season.

Plant 3 (§ 21), 1929. On May 3rd, two bines, 4 ft. high, were present. One of these was cut down to provide a stock for one scion from the chlorotic plant AA. 4. The scion was only 3 in. long and showed no chlorotic symptoms. No disease symptoms appeared subsequently on the scion, which by July 31st reached a height of 4 ft. The bine of the stock plant remained healthy and reached over 6 ft. in height.—1930. On April 12th, on the one bine present, suspicious symptoms were noticed on the leaves at the 6th node and upwards, and by April 15th the presence of the chlorotic disease was established beyond doubt. The bine was then 3 ft. high, with 10 nodes. The chlorotic symptoms continued to appear in the later leaves and were present throughout the season.

Plant 4 (§ 22), 1929. On May 3rd, four bines were present, the longest of which (4 ft.) was cut to provide the stock for one scion from the chlorotic plant AA. 4. The scion was 3 in. high, with only two pairs of very small leaves expanded and these showed no symptoms of chlorosis. By July 30th, the scion was 21 in. high with 10 nodes; the leaves were green and normal in appearance, without symptoms of chlorosis. The stock plant remained healthy, with two bines 6 ft. high.—1930. On April 12th, the leaves on the one bine produced were pale green, though the symptoms of chlorosis were absent. By April 28th, this bine (now 6 ft. 2 in. high) showed symptoms of the Nettlehead disease, and this was confirmed during subsequent growth. It is unknown how this infection occurred.

With three out of the four stock plants grafted, therefore, there was clear evidence of the transmission of the chlorotic disease from the scion, although no symptoms became apparent until the second season. With the fourth plant, the experiment was nullified by the unexplained appearance of the Nettlehead disease.

## 2. BUDDING.

Brief mention was made in our former paper (p. 246) of experiments with budding, in which no positive results were obtained in 1929, but the method used was not described. Since, as recorded below, positive results were obtained in 1930, with the plants budded in 1929, details concerning the method are now given. June 1st, 1929. One chlorotic leaf, the bud in its axil, and a shield-shaped piece of the bine, in all cases taken from the same chlorotic plant (Ch. 6)<sup>1</sup> was bound with india-rubber tape on to a suitably cut area on the side of one bine of the plant to be inoculated. Nine plants were inoculated, comprising one Fuggles (derived from a hop-garden "C" in Worcestershire, seven plants received from the Continent under the name of "Mid-European Golding"<sup>2</sup> and one Seedling Variety, Ref. No. OK. 50, raised by crossing the Late Bavarian German variety with an English male hop. The shield, with bud and leaf, was bound on at one side of a node where it replaced a corresponding shield, bud, and leaf removed from the healthy plant. The bine on which this operation was performed was cut off 1 in. above the budded node. The node chosen was commonly about  $3\frac{1}{2}$  ft. from the ground. The healthy plants used were growing in the Experimental Hop Garden and were strong and vigorous. The budding was done on a cool evening, and subsequent showery weather probably favoured the inserted parts and helped to keep the leaves and the buds alive. Ten days later, the chlorotic leaves were not wilted but appeared as fresh and turgid as though they were growing on that bine; eventually, however, they withered and fell off. In most cases, even though the inserted bud made but little growth, the shield remained alive, and it is of interest to note (see Table I) that in all three cases where the shield failed to unite, no transference of the disease was effected. In the first season (1929) no symptoms of disease appeared on any of the budded or control bines in the hills. The control (untreated) bines were all vigorous and grew to the top wire (12 ft.) or over. During 1930 the budded plants were frequently inspected but not at regular intervals; the dates given in Table I indicate the times at which the chlorotic symptoms were first observed. In all the four plants of the "Mid-European Golding" in which symptoms developed, all the bines of each plant became affected, though their

<sup>1</sup> One of the diseased Fuggles plants originally obtained from Worcestershire in 1927.

<sup>2</sup> Two of these (Ref. nos. OK. 81, OK. 94) originated from Saaz and were obtained from Dr C. Blattay in 1928; and five (Ref. nos. OK. 96, OR. 91, OT. 82, OY. 39, OX. 18) were sent by Dr P. Vrbováky from Yugo-Slavia in 1928. The variety appears to be identical with Fuggles.

growth was not appreciably hindered, the shortest of the twelve bines produced being 11 ft. high, and many were hanging 4 ft. over the top wire (representing a total height of 16 ft.), when measured on August 4th, 1930.

Table I.

*Hop plants inoculated with chlorotic buds, June 1st, 1929.*

Ref. nos. of plants budded	Where budded (height above ground) ft. in.	July 2nd, 1929, state of inserted		July 29th, 1929, state of inserted		Chlorosis observed Date 1930	August 4th, 1930	
		leaf	bud (in. long)	leaf	bud (in. long)		Total no. of bines	No. of bines showing chlorosis
New Seedling: OK. 50	3 10	Dead	‡	Dead	2‡	Aug. 4	3	2
Mid-European Golding:								
OK. 81	3 5	Alive	‡	Dead	1	May 21	3	3
OK. 94	3 9	Dead	‡	Dead	‡	May 28	4	4
OK. 96	4 2	Dead	‡	Dead	Dead*	Healthy	3	Nil
OR. 91	3 10	Dead	Dead†	Dead	Dead*	Healthy	3	Nil
OT. 82	3 9	Dead	Dead	Dead	Dead*	Healthy	4	Nil
OY. 39	4 1	Dead	1	Dead	3	May 21	3	3
OX. 18	3 8	Dead	‡	Dead	Dead†	May 28	2	2
Fuggles:								
§ 4 = OZ. 35	3 6	Dead†	‡†	Dead	Dead†	May 29	2	2

\* The whole inserted shield and bud was dead and easily removed.

† The shield was still alive, and minute lateral buds were alive on each side of the dead bud.

‡ The date of this observation was July 16th.

The seedling hop OK. 50 examined on May 28th, 1930, when its three bines were 6-8 ft. high, showed no chlorotic symptoms; on August 4th<sup>1</sup> the three bines were 6 ft. over the top wire, and on two of these the leaves were chlorotic to a height of 9 ft.

The Fuggles plant inoculated was a pot-plant; in addition to the one bine which was budded, there were two which grew to a height of over 10 ft. The leaf and bud inserted both died, and on July 29th, 1929, only the lower part of the shield (which had united) was alive. During the winter of 1929, this plant was removed from the pot and was planted in the hop-garden (Ref. No. OZ. 35). On May 29th, 1930, chlorotic symptoms became apparent; the two bines (both of which showed chlorosis) were 8 ft. and 14 ft. in height.

Five further pot-plants of Fuggles (§§ 28-32), grown from rooted sets obtained from plants originating from a grower in Worcestershire<sup>2</sup>, were

<sup>1</sup> It is probable that the disease became evident during June or July, but the plant was not inspected during those months.

<sup>2</sup> Four of these plants were derived from a hop-garden "A," and one (§ 31) from a hop-garden "B."

budded with chlorotic buds on May 21st, 1929. The same method was used as in the other experiments; the bine to be operated upon was cut off above a node at about 3 ft. from the soil level and the chlorotic bud was inserted on one side of the uppermost node. All the plants during that season (1929) were vigorous, and produced, in addition to the bine inoculated, either two or three bines 6 ft. high. The first three chlorotic buds to be used were obtained from a diseased plant (Ch. 4), and the remaining two from a diseased plant (R. 3/8). Both these diseased plants were sent from Worcestershire in 1927, and were growing in the Experimental Hop Garden at Wye. As is shown in Table II, no chlorosis appeared in the first season, but in 1930 symptoms were developed while the plants were kept in an unheated glasshouse, the earliest being observed on March 24th. In Table II are recorded the earliest dates at which symptoms were discovered and the number and height of bines on each plant which were healthy or diseased at that time. As growth continued, all the bines became chlorotic, the most advanced symptoms being shown. By the end of the season the bines had reached a height of 8–10 ft.; the laminae at the nodes nearer the ground, although chlorotic, were of normal size, but higher up the bine they became smaller and smaller until they became almost rudimentary. The leaves fell early (when the laterals were starting to grow out) and as a result, by September, the laterals on the long bare bines were the only parts of the plant with leaves. The laterals commonly bore chlorotic leaves at the basal 2 or 3 nodes, the remaining leaves being healthy. Two or three laterals on each bine bore healthy leaves.

Table II.

*Pot-plants, variety Fuggles, each budded with one chlorotic bud, May 21st, 1929.*

Exp. No.	Source of chlorotic bud	Height at which bud inserted (in.)	July 16th, 1929, condition of		Date symptoms first observed†	1930	
						Height (ft.) of bines on that date	
			Bud	Shield		Healthy	Chlorotic
§ 28	Ch. 4	37½	Dead	Alive*	April 21	9½, 9, 5	6½
§ 29	Ch. 4	36	½ in.	Alive*	April 2	None	3½, 3½
§ 30	Ch. 4	30	Dead	Alive	April 20	None	6, 5½, 3
§ 31	R. 3/8	36	Dead	Alive	March 24	½, ½	1½
§ 32	R. 3/8	36	Dead	Alive‡	—	4, 4, 4, 4	None

\* Only the lower part of the shield, below the dead bud, representing an area of about 1.5 × 0.4 cm., was alive and green.

† The plants were in an unheated glasshouse until May 7th, 1930.

‡ Although alive on July 16th, the union of the shield was rendered ineffective through death of the bine down to the node below that at which the bud was inserted. This occurred between July 16th and 30th, 1929.

Only one of the five plants failed to contract the disease. On this plant, the inoculated bine died back to a node below the one at which the chlorotic bud had been inserted. Although the shield was known to have united and to have been alive from May 21st to July 16th, the virus apparently had insufficient time to travel down the bine before the dying back occurred between July 16th and July 30th.

### 3. RUBBING LEAVES WITH MACERATED TISSUE.

On June 3rd, 1929, four plants, received in 1928 under the name of "Mid-European Golding" from Dr Pavel Vrbovský, Yugo-Slavia, and planted in the autumn of 1928 in the Experimental Hop Garden at Wye, were inoculated in the following manner. A number of leaves showing chlorotic symptoms were obtained from a diseased plant<sup>1</sup> and were ground to a moist pulpy mass in a mortar which previously had been thoroughly cleaned with an abrasive soap preparation and washed with hot water. A little distilled water (less than 5 c.c.) was added and mixed with the pulpy mass by means of the pestle used for the grinding. Of the four vigorous plants inoculated, two (Ref. Nos. 358 *a*, 359 *a*) had two bines, and two (Ref. Nos. 327 *a* and 330 *a*) had six bines each. One leaf at two adjacent nodes on one bine of each plant was rubbed with the fingers moistened with the inoculum. Friction over most of the area of the lamina was caused by using the thumb and forefinger, but it was found that less damage to the leaf was caused if the whole lamina was inverted on the palm of one hand and rubbed on the ventral surface with the moistened finger tips of the other hand. All the eight leaves were considerably bruised and the tissues of the laminae were here and there torn, but the leaves were not damaged so much as to prevent their recovery of a normal appearance after a short time. Small fragments of the ground-up pulp adhered to the bruised laminae for some days. All the bines on the plants chosen had reached a height of about 12 ft. on June 3rd, and on each of the four bines on which leaves were rubbed the lower leaf selected was at the height of about 5 ft. from the ground, the upper leaf being about 6 in. higher. No symptoms of disease developed on any of the 16 bines under observation during the season of 1929, and normal "burr" appeared on July 29th, followed by a crop of cones.

Early in the following season (1930) chlorosis appeared in all of the four plants, as follows. 358 *a*: First appearance May 28th, on three bines; by August 4th, all the four bines showed chlorosis. These bines, ranging in height from 11 ft. to 16 ft., showed the symptoms in all leaves

<sup>1</sup> One originally obtained from Worcestershire in 1927 (Ref. No. Ch. 20).

except those in the upper parts. The topmost 4 ft. or even 6 ft. bore healthy green leaves, and in the case of one of the bines (11 ft. high) the leaves were chlorotic only at nodes between 2 ft. and 3 ft. from the ground. This formation of healthy foliage in the upper part of the bine suggested that perhaps the rate of growth of the plant or some such factor as temperature caused a sudden suppression of the symptoms. Such an occurrence is common with the laterals on chlorotic plants, the lowermost leaves (next to the main stem) being frequently the only ones to exhibit symptoms. 359 *a*: First appearance May 28th, on five bines. Two of these bines were, by August 4th, 12 ft. high and bore leaves with the most pronounced symptoms up to a height of 7 ft. and 8 ft. respectively, the chlorotic leaves being much distorted and becoming smaller and smaller as the height increased. Suddenly, on both bines, above the 7 ft. or 8 ft. level, the leaves were developed normally and appeared green and healthy to the very tip of the bine. 327 *a*: First appearance, May 28th, on four bines. By August 4th, all six bines, 10 ft. to 14 ft. high, were chlorotic. The laterals showed all stages from complete chlorosis of a long (3 ft.) lateral to chlorosis only of basal leaves, and a few laterals were healthy. 330 *a*: First appearance, June 24th, on 6 bines 12 ft. high.

It is somewhat remarkable that no symptoms of disease appeared in the bines produced in the same season when the inoculations were made. It is inconceivable that the virus took only a downward course to the rootstock (whence the diseased bines of the following season arose); it would seem therefore that the amount of virus present in the bines during the first season was insufficient for the production of disease symptoms.

If we summarise the experiments given above and in our former paper, we find that the chlorotic disease of hops has been successfully transmitted by grafting (6 times), by budding (11 times) and by juice rubbed on wounded surfaces (4 times). The two other virus diseases of the hop, mosaic and nettlehead, are, so far as is known, transmissible only by the process of grafting. By virtue of the infectivity of the juice, this disease takes its place among the similarly infectious virus diseases of tobacco, tomato and potato. By reason of this character, it appears very probable that the chlorotic disease of hops would be spread by workers in the hop garden employed in the cultural operations of stripping the bines of the lower leaves, and possibly also in "dressing" (pruning) the rootstock.

## SUMMARY.

1. Further experiments are recorded in which the transmission of the recently described chlorotic disease of the hop was obtained by means of grafting and budding.

2. Transmission was obtained also by rubbing healthy leaves with macerated chlorotic tissue in such a way that bruising of the lamina resulted.

3. In all the experiments described, with one exception (see above, p. 6), no symptoms of disease appeared until the year following that in which the inoculations were made.

*(Received May 6th, 1931.)*



## A DISEASE OF THE ARUM LILY CAUSED BY *PHYLLOSTICTA RICHARDIAE*, N.SP.

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(With Plate I.)

IN April, 1930, Mr W. C. Moore called my attention to a serious disease of the Arum Lily (*Richardia africana*) in the Scilly Isles, where this plant is grown out-of-doors on a large scale for the sale of the blooms. Through the kindness of Mr Gordon W. Gibson, Director of the Isles of Scilly Experimental Station, specimens of this disease were sent to me at intervals during the summer of 1930, on which this investigation has been based. I am much indebted to Mr Gibson for information about the conditions under which the disease occurs in the Scilly Isles. He states that it is most severe in spring and early summer during periods of high humidity and moderate temperature. In March, 1931, Mr L. Ogilvie, of the Long Ashton Research Station, sent me specimens of the same disease on Arums grown under glass at Yeovil, Somerset. In the *Report on the Occurrence of Fungus, Bacterial and Allied Diseases of Crops in England and Wales for 1925-27* (Ministry of Agriculture and Fisheries, Miscellaneous Publications, No. 70) this disease is also reported from other parts of the country.

The disease appears in the form of brown blotches of varying size on the leaf laminae, petioles, stalks of the inflorescences and the white spathes (Figs. 1-3, Plate I). On the leaf lamina and petiole the brown spots, when young, are often surrounded by a narrow yellow zone. The blotches may extend so much that practically the whole of the leaf is destroyed. Sometimes the central parts of the blotches fall away, leaving holes. On the petioles and stalks of the inflorescences the lesions, which are usually confined to one side, are shrunken. If the diseased tissues are kept under moist conditions innumerable, small, brownish pycnidia of the *Phyllosticta* type develop. It seemed likely, therefore, that a species of *Phyllosticta* was the cause of the disease.

Pure cultures of this fungus from the Scilly Isles were established from single spores on a variety of media. The same fungus was subsequently isolated from the diseased material sent from Somerset. Pycnidia developed in great abundance on Dox's agar medium, and spores from

this source were used for inoculation experiments. In the first series of inoculations the leaves of healthy Arum plants were sprayed with a dense spore emulsion by means of an atomiser, some of the leaves being previously wounded; the plants were then covered with bell jars for 48 hours. None of the leaves thus inoculated became infected. Meanwhile information had been received from Mr Gibson that the disease spread in the Scilly Isles only under very humid atmospheric conditions, and it had also been noticed that in hanging-drop cultures few spores germinated within 48 hours. In the next series of inoculation experiments, therefore, bell jars were kept over the plants for 7 days, the inoculations being carried out as before. Eleven days after the commencement of these experiments numerous brown spots of irregular shape were present on leaf laminae which had been inoculated without wounding. These spots spread rapidly, and several of them were surrounded by narrow, yellow zones, as in the naturally occurring diseased material. Although the disease did not develop at any of the wounds on other leaf laminae it did arise in association with wounds on the petioles which had been sprayed with the spores. These lesions on the petioles were precisely similar to those on the material sent from the Scilly Isles. Other leaves which had been sprayed with water only and which had been kept under the same conditions remained healthy. Subsequently, pycnidia developed on the brown blotches in the leaf laminae and petioles, and from them the same fungus was obtained in culture as that with which the inoculations had been made.

The results of these experiments indicate that a species of *Phyllosticta* is the cause of this serious disease of the Arum Lily. The fungus is a real parasite, as it can infect unwounded tissues provided that the atmospheric environment is sufficiently humid. The successful inoculation experiments were carried out in an unheated greenhouse, with temperatures fluctuating between 13 and 24° C. (55°–75° F.).

There are several previous records of the occurrence of a species of *Phyllosticta* or *Phoma* on leaves of the Arum Lily. In 1894 Halsted<sup>(1)</sup> in a paper on "Diseases of Calla" wrote as follows: "The Calla leaves sometimes become blighted with large, ashy spots upon which are minute pimples of a dark colour. The fungus causing the loss of green colour in these areas is *Phyllosticta Richardiae* Hals. As this fungus is close of kin with many others of its genus, causing leaf spots upon various cultivated plants, little further need be said." Halsted gave no diagnosis of this fungus and apparently carried out no inoculation experiments with it.

In 1913 Ritzema Bos (4) referred to a species of *Phyllosticta* causing leaf spots on Callas at Zutphen, Holland, but again no serious investigation of the disease was carried out.

In 1913 also Mercer (2) published a full account of *Phoma Richardiae* n.sp. which he found on decaying parts of Calla leaves (Arum Lily). He concluded, however, that this fungus was not parasitic, as all his inoculation experiments gave negative results. Although Mercer's fungus is closely related to the fungus under consideration in this paper there are certain important morphological differences between them. For instance, Mercer states that on plum agar brown conidia of the *Alternaria* type are formed on the aerial mycelium. On this medium, made up according to Mercer's formula, no such conidia were formed by my fungus. On the other hand, both fungi have peculiar brown, thick-walled, much septate, irregular aggregations in old hyphae, especially when embedded in the medium, which Mercer refers to as "gemmae." In Mercer's fungus the pycnosporos have 2-3 oil drops towards each end, but in my fungus these are generally absent. There are minor differences in the dimensions of the pycnidia and pycnosporos of the two fungi.

In view of the absence of any diagnosis of *Phyllosticta Richardiae* Hals., and in view also of Mercer's results, it seems best to name the fungus now under consideration as *Phyllosticta Richardiae* n.sp., the diagnosis being as follows:

*Phyllosticta Richardiae* n.sp. Pycnidia roughly spherical, brownish black, 120-180 $\mu$  in diameter; ostiole circular in outline, 20-30 $\mu$  in diameter, sometimes excentric; pycnosporos unicellular, hyaline, oval, often pointed at one end, 3.7-7.3 $\mu \times$  2.7-3.7 $\mu$ , average 5.5 $\mu \times$  3.5 $\mu$ , exuding as small greyish tendrils. Parasitic on the leaves and inflorescences of *Richardia africana*, causing large brown blotches.

The cultural characteristics of the fungus on the following media in Petri dishes are:

*Dox's agar.* Much aerial mycelium, white when young and persisting so for a long time, then greyish; old mycelium in the medium somewhat darker.

Pycnidia formed rapidly in great abundance, scattered irregularly in the medium, sometimes compound.

*Plum agar* (Mercer's formula (2)). Aerial mycelium fairly abundant, white when young, rapidly becoming greyish brown; old mycelium in the medium very dark; old hyphae forming thick-walled, much septate, irregular aggregations.

Pycnidia fairly abundant, scattered, sometimes compound.

*Arum Lily agar.* Very little aerial mycelium, white when young, becoming slightly darker; old mycelium in the medium almost hyaline.

Pycnidia abundant, scattered, sometimes compound.

A culture of Mercer's fungus was obtained from the Centraal Bureau voor Schimmelcultures, Baarn, and the differences between it and my fungus justify their separation.

The control of this disease under glass should not be difficult. In view of a very high atmospheric humidity being necessary for infection the greenhouses should be well ventilated so that the humidity never becomes unduly high. Diseased parts of plants should be cut off and destroyed as soon as seen. The fungus may be able to live saprophytically upon dead Arum Lily debris and possibly in that way survives from season to season; it is important, therefore, that all such material should be promptly burnt.

Control of the disease where Arum Lilies are grown out-of-doors, as in the Scilly Isles, is more difficult. Here also, however, every effort should be made to destroy promptly diseased foliage and blooms, and also all dead parts as the Arum Lilies die down. If possible, some rotation in the growing of these plants should be practised, and Mr Gibson states in a letter that he is "under the impression that the disease is largely eliminated by planting the corms after drying in a new area." It has been shown by Metcalfe<sup>(3)</sup> that *Phoma Lavandulae*, the cause of a serious disease of lavender, is able to live saprophytically upon the dead remains of a common weed, *Chenopodium album*. It may be that in some such way *Phyllosticta Richardiae* also persists from season to season. Although, as Mr Gibson points out, actively growing Arums choke all weeds, these begin to develop as the plants die down. As far as possible land used for Arums should be kept free from weeds throughout the year.

In conclusion I wish to thank Mr W. C. Moore for assistance with the references on this disease, and Dr A. Fikry and Mr T. A. Russell for help in carrying out the inoculations.

#### SUMMARY.

1. A serious disease of the Arum Lily (*Richardia africana*) is described, which causes the formation of large brown blotches on all parts of the aerial shoot system.

2. The cause of the disease is *Phyllosticta Richardiae* n.sp., a diagnosis of which is given.

3. Suggestions are made for the control of this disease both under glass and in the open.

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## EXPLANATION OF PLATE I.

- Fig. 1. Blotch on Arum Lily leaf caused by *Phyllosticta Richardiae* n.sp.  $\times \frac{1}{2}$ .  
Fig. 2. Arum Lily leaf badly attacked by *Phyllosticta Richardiae* n.sp.  $\times \frac{1}{2}$ .  
Fig. 3. Petiole of Arum Lily with lesion caused by *Phyllosticta Richardiae* n.sp.  $\times \frac{1}{2}$

(Received May 20th, 1931.)



Fig 1



Fig 2



Fig 3.

BROOKS.—A DISEASE OF THE ARUM LILY (pp. 16-20).



# *FUSARIUM* SPECIES ON BRITISH CEREALS

## THE GIBBOSUM GROUP. I. *F. SCIRPI* LAMB. ET FAUTR.

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(With Plate II and 2 Text-figures.)

THE present paper is a continuation of the author's investigations of the species of *Fusarium* occurring on British cereals<sup>1</sup>. The species *F. Scirpi* Lamb. et Fautr. was originally described in detail by Appel and Wollenweber<sup>(1)</sup> under the name of *F. gibbosum*, the description being of its pseudopionnotal form only. Atanasoff<sup>(2)</sup> mentions the occurrence of aerial mycelium but gives no details. The writer obtained a mycelial form of the organism first from the basal parts of *Triticum monococcum*<sup>2</sup> and later from barley roots from two localities in the Eastern Counties. A new variety of *F. Scirpi* was also discovered on the bases of barley grown at Woburn<sup>3</sup>. In the absence of previous records these two forms could not then be identified. The subsequent investigation revealed difficulties in developing a distinctive form from the mycelial type, and in the puzzling variations in monospore cultures of the new variety. These difficulties are now removed, the relationship of the varieties elucidated, and the pathogenicity of the species towards cereals ascertained. It being now necessary to distinguish two varieties the old one is here designated *pallens* and the new one *nigrans*.

### *F. SCIRPI* VAR. *PALLENS*.

*The mycelial form.* Single-conidium cultures from naturally infected material yielding this mycelial form showed the following characters:

*Aerial mycelium.* At first white and abundant on all artificial substrata, loose on starchy, more compact or woolly on sugar and glycerine media; developing ochraceous tawny<sup>4</sup> shades after 1 month and subsequently patches of Isabella and olive

<sup>1</sup> Previous papers have appeared in the *Ann. App. Biol.* xv, 213, 1928; xvii, 43, 1930; xviii, 158, 1931. The methods and media used are there described.

<sup>2</sup> Supplied by Mr W. A. R. Dillon Weston, School of Agriculture, Cambridge, 1929.

<sup>3</sup> Supplied by Mr W. C. Moore, Plant Pathological Laboratory, Harpenden, 1929.

<sup>4</sup> References to Ridgway's colour standards throughout.



lake, with more distinctive shades of olive lake to olive citrine on glycerine media; the mycelium next the substratum forming a dense, cinnamon-tinted layer.

*Substratum.* Wheat-meal agar becomes pale sayal brown, and hard oat agar pale wood brown<sup>1</sup> in 3 months or longer; glycerine agar, distinctive shades of olive ochre, old gold, to olive lake after 2-3 months.

*Chlamydospores.* Abundant in aerial mycelium, very rare or absent in conidia; terminal and intercalary, commonly in chains or clusters often of twelve to twenty; smooth walled; up to  $15\mu$  in diameter, or  $13 \times 18\mu$ ; at first cinnamon tinted, then cinnamon colour, and later olive brown in the olive lake parts of the mycelium. Sometimes aggregations of chlamydospores form sclerotial structures, 0.5-3 mm. in diameter, and from honey yellow to dark greenish olive in colour. The change of colour of the aerial mycelium is due partly to development of colour in the hyphae, but mainly to the increase in number of chlamydospores and development of darker shades in them.

*Sporodochia.* First appear on the sclerotial aggregates on wheat-meal and hard oat agars in cultures from 2 to 3 months old; pale ochraceous buff to pinkish buff; after 4-6 months, clusters from 2 to 4 mm. high and of light ochraceous salmon colour occur in the shrunken bases of media slants also; very rarely on aerial mycelium except where partly torn previously.

*Conidia.* On aerial mycelium abundant, but of varied shapes and not characteristic (Text-fig. 1, I). 3-septate, from 85 to 90 per cent.,  $31-39\mu \times 2.8-4.3\mu$ , average  $33.3 \times 3.6\mu$ . 4-septate occasional, 5-septate very rare. In young sporodochia 5-septate predominate, sometimes up to 75 per cent.,  $39-52\mu \times 4.0-5.2\mu$ , average  $45 \times 4.7\mu$ . 6- (rarely 7-) septate, 1 per cent., average  $51.1 \times 5.0\mu$ . 3-septate 20 per cent.; 4-septate 3 per cent. These sporodochial macroconidia, though not fully characteristic, closely resemble the typical forms (Text-fig. 1, II), and sub-cultures from these yield the pseudopionnotal form.

Direct transfer of aerial mycelium to natural or artificial substrata reproduces the mycelial form, but with some reduction in coloration of mycelium, number and colour of chlamydospores, and sclerotial aggregates; the general features, including the development of sporodochia, remain as previously described. In cultures derived from microconidia a similar slightly modified mycelial form arises. Differences of temperature influence the rate but not the type of growth. Identification of the species when in the mycelial form is, therefore, a slow process, and sub-cultures along the lines mentioned delay or render it impossible. The proportion of macroconidia on the aerial mycelium increases slightly after several weeks, and sub-cultures from such mycelium, repeated if necessary, show suppression of aerial mycelium, reduction of chlamydospores, absence of sclerotial aggregates, and development of incomplete pseudopionnotes. Further, aerial mycelium, or single microconidial growths, transferred to hard oat agar in Petri dishes sometimes

<sup>1</sup> Never Dresden to mummy brown, as recorded by Atanasoff (2), p. 77; but see p. 27 of this paper.

give rise to the pseudopionnotal form in sectors, from which similar cultures may be obtained. Such sub-cultures are, however, still in sub-normal condition, the conidia being predominantly of micro-type, whilst the macro-type generally lacks the distinctive curvature, the elongate apical segment, and the prominent foot of the normal type. *F. Scirpi* in this condition cannot be distinguished with certainty from *F. ossiculum* Berk. and Br. The normal, pseudopionnotal form that exhibits clearly the diagnostic features of *F. Scirpi* is, however, readily obtained from single macroconidia or mass transfers from the sub-normal type.

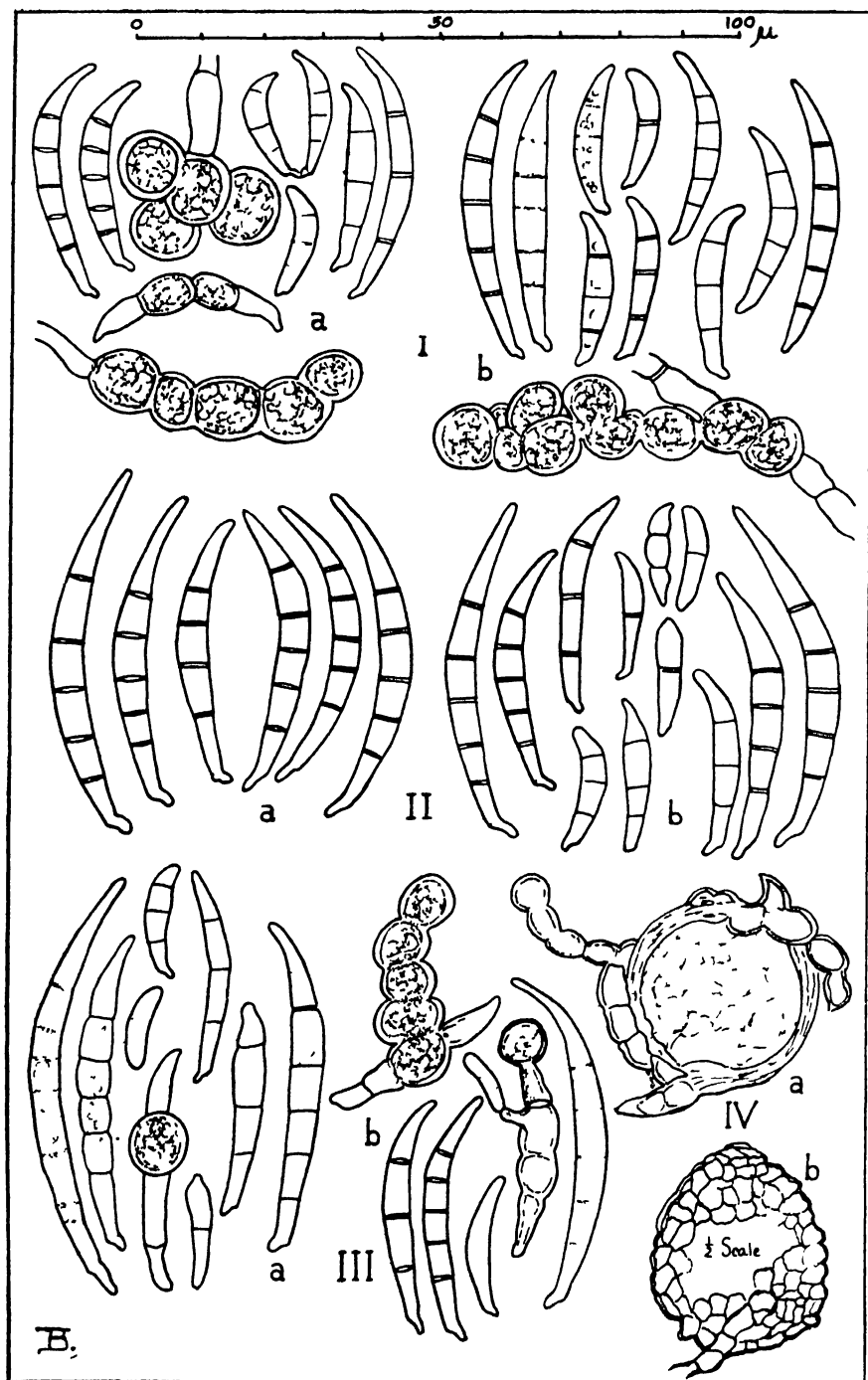
To change the pseudopionnotal to the mycelial form is a more difficult matter, and probably explains the absence of records of this phase in former investigations. Neither promising sectoral growths nor other sources of inoculum that originate from the pseudopionnotal form in vigorous condition yield the mycelial form. The change may be accomplished by transfer of plectenchymatic mycelium from old cultures in which the conidia have lost vitality (*e.g.* from 12 to 18 months old); subsequent selection of sectoral growths and final cultivation on natural substrata yields the primary mycelial form.

*The pseudopionnotal form.* This form arises from macroconidia, and as these spores prevail on most naturally infected materials when incubated the pseudopionnotal form is the one commonly obtained from such sources. Its development in culture from the mycelial form is described above. The characteristics of the pseudopionnotal (or "normal") form are as follows:

*Aerial mycelium.* Typically none, except a small tuft at the upper extremity of media slants, straw, etc., but on hard oat agar a sparse, loose, floccose growth, white to cinnamon tinted, occurs on the upper part of slants. On hard oat and hard potato dextrose agars hyphae pierce the pseudopionnotal layer and form thin, superficial patches on old cultures.

*Pseudopionnotes.* Originating as minute sporodochia deposited in zonate manner on sparse, delicate hyphae radiating on the surface of media from the point of inoculation; the hyphal stroma later disappears, leaving the minute, closely packed but non-convergent sporodochia as a moist layer on the submerged plectenchyma. On wheat-meal agar, from pale ochraceous salmon to pinkish cinnamon, remaining so for months; on hard oat and hard potato dextrose agars, from pinkish to vinaceous cinnamon (1 month), then cinnamon, and eventually sayal brown (3 to 6 months); on glycerine agar, thin and incomplete, faintly ochraceous salmon to vinaceous cinnamon or pinkish cinnamon (3 to 6 months).

*Substratum.* Wheat-meal agar, from pale cinnamon (3 months) to pale clay (6 months); hard oat agar, the plectenchymatic layer from Mikado to Verona brown (3 to 6 months), the deeper parts avellaneous, wood brown to buffy cinnamon (6 months); glycerine agar tinted faintly as the pseudopionnotes. Rice, grenadine to bittersweet pink, the colour being slightly reduced in intensity by alkaline solutions,



and not changed by acids<sup>1</sup>. Straw, in moist chambers, bears non-converging sporodochia with a few superficial linkage hyphae and a tuft of hyphae at the upper extremity; the conidia from these sporodochia are more uniform and characteristic than those from artificial media (Text-fig. 1, II).

*Chlamydospores*. Always abundant, but relatively fewer than in the mycelial form and never as sclerotial aggregates. In hyphae, in chains and clusters; in conidia, terminal or intercalary, one or more, sometimes small clusters of three or four; usually from 9 to 10.5  $\mu$  but frequently 13  $\mu$  in diameter. Smooth walled, at first faintly tinted, later darkening to cinnamon or cinnamon brown after many months, hence the slow change of colour of cultures.

*Conidia*. Dorsal curvature from hyperbolic to parabolic; conspicuously broader near the middle; the apical segment elongated and pointed, the basal segment with a prominent foot; septa typically 5, slightly closer near the mid-septum when the curvature is pronounced; some 6- and 7-septate on most agar media and on potato (Text-fig. 1, III).

(1) From sporodochia on sterile wheat straw (Text-fig. 1, II): 3-septate, 14 per cent., average  $38.3 \times 3.5 \mu$ . 4-septate, 6 per cent., average  $45.1 \times 4.3 \mu$ . 5-septate, 80 per cent.,  $39.57 \times 3.9-5.0 \mu$ ; average  $49.9 \times 4.5 \mu$ . 0-, 1-, 2-septate very rare; more than 5-septate absent.

(2) From pseudopionnotes on wheat-meal agar, culture 4 weeks old; mainly of characteristic shape but contents granular and septation masked; apparently 5- to 7-septate;  $47-65 \times 4.0-5.2 \mu$ , average  $56.6 \times 4.6 \mu$ .

(3) From pseudopionnotes on hard oat agar, culture 4 weeks old; condition as on wheat-meal agar (Text-fig. 1, III);  $49.5-65 \times 4.4-5.0 \mu$ , average  $59.5 \times 4.7 \mu$ .

(4) From pseudopionnotes on cooked potato plug (Text-fig. 1, III): 3-septate, 5 per cent., average  $37.7 \times 4.2 \mu$ . 4-septate, 4 per cent., average  $39.9 \times 4.5 \mu$ . 5-septate, 64 per cent., average  $47.5 \times 4.8 \mu$ . 6-septate, 3 per cent., average  $54.6 \times 5.0 \mu$ . 7-septate, 0.5 per cent., average  $55.5 \times 5.1 \mu$ . The septation mode and length of conidia differ to some extent from those given by Appel and Wollenweber (1) for this substratum, but there is agreement in width<sup>2</sup>.

<sup>1</sup> The coloration of hard oat agar, and the colour and its reaction on rice, are amongst the features distinguishing this variety from the variety *nigrans* (p. 27); the coloration of glycerine agar differs from that produced by the mycelial form (p. 22).

<sup>2</sup> Dr Wollenweber kindly verified the species here dealt with; the variations mentioned are normal within a species, such differences arising from age of culture, humidity of atmosphere, etc.

Text-fig. 1. *F. Scirpi* var. *pallens*.

- I. Conidia from natural material, showing varied shape and size: (a) from aerial mycelium on barley ear; (b) from first aerial internode of mature wheat after incubation.
- II. Conidia from sterile straw, culture 6 weeks old: (a) from sporodochia, which yield a uniform normal type; (b) from spore clusters in the tuft of aerial mycelium.
- III. Conidia from pseudopionnotes, showing the various forms: (a) from hard oat agar, culture 4 weeks old; (b) from cooked potato plug, culture 6 weeks old.
- IV. Perithecial structures: (a) early stage, before development of the peridium; (b) later stage ( $\frac{1}{2}$  scale size).

(5) From pseudopionnotes on hard potato agar, culture 4 weeks old; a large proportion abnormal, with barrel-shaped segments, and a higher septation mode; 0-, 1-, 2-septate occasional; 3-septate 13-23, 4-septate 10-14, 5-septate 45-80, 6-septate 10-16 per cent.; 7-, 8-, 9-, 10-septate occasional, up to  $65 \times 5.3 \mu$ , but usually swollen and wider than this.

*Perithecial structures.* Incipient perithecia are common in cultures of this variety, *pallens*, but more so and better developed in the variety *nigrans*. They appear after about 2 months in cultures derived from pseudopionnotal or sporodochial (not mycelial) sources, on potato, straw, barley ears, etc., and amongst the floccose mycelium as well as embedded in the dry tip of various agar media slants. In the early stages they are nearly colourless or tinted cinnamon; older ones vary from greenish blue to deep blue-violet, but frequently a cinnamon or cinnamon-brown colour predominates. In structure they resemble the perithecia of *Gibberella Saubinetii*. In the variety *pallens* they attain a size of  $120 \times 150 \mu$ , whilst in the variety *nigrans* they reach  $200 \times 220 \mu$  under parallel conditions. Production of perithecia is favoured by normal room temperature ( $16-18^{\circ} \text{C}$ .) and variation, but they have not matured further than the development of finely granular contents, without trace of asci, on any substrata under any of the various environmental conditions tried up to periods of 9 months' duration (Text-figs. 1, IV; 2, IV).

*F. SCIRPI* VAR. *NIGRANS* (n.v.).

Single-conidium cultures prepared from the natural material previously mentioned (p. 21) showed the characters described below for the pseudopionnotal stage; no mycelial form has been obtained from naturally infected material.

*The mycelial form.* This has been obtained in cultures from single conidia on living seedlings of wheat and barley grown in artificially contaminated soil. It is of rare occurrence and has not been produced at will. It shows no distinctive difference from the mycelial form of the variety *pallens*, although there is a more obvious tinge of pinkish cinnamon in the aerial growth and in the plectenchymatic layer during the first weeks of growth. Cultures from aerial mycelium and from mycelial microconidia, and the eventual production of sporodochia and pseudopionnotes, follow the lines previously described; in these higher stages the characteristic features are as follows:

*Aerial mycelium.* As described for the normal condition of variety *pallens* (p. 23), except that the floccose growth on hard oat agar becomes loaded with sporodochia, and on hard potato dextrose agar there is scanty, cottony, cinnamon-tinted growth from the first.

*Pseudopionnotes*. Developed after the manner of the variety *pallens* but more rapidly. On wheat-meal agar pinkish cinnamon, buckthorn to Dresden brown, cinnamon to sayal brown successively in 3 weeks, to Prout's brown afterwards and remaining so. On hard oat agar pinkish cinnamon to cinnamon, cinnamon brown, Prout's brown successively in 3 weeks, and subsequently from mummy brown to bister or sepia. On hard potato dextrose agar buckthorn to Dresden brown at 2 weeks, followed by Prout's brown and mummy brown or bister. On glycerine agar ochraceous buff to Prout's brown at 3 weeks, and mummy brown by 2 months.

*Substratum*. Wheat-meal agar, Prout's brown in the plectenchymatic layer to deep honey yellow below in 2 weeks; and in 2 months from dark vinaceous or Hay's brown near the base to taupe or light seal brown above. Hard oat agar, mummy brown or warm sepia in the plectenchymatic layer, with Mikado brown or russet below, in 3 weeks; and at 2 months light seal brown and dark vinaceous or taupe brown respectively. Glycerine agar, from faint olive ochre at 2 weeks, to Saccardo's umber at 2 months. Hard potato dextrose agar, Dresden or Prout's brown at 3 weeks, and later dark chocolate. Rice, at first bittersweet pink, is cinnamon rufous after 2 weeks; this colour is not changed by dilute acid, but alkali intensifies it to mummy and chocolate browns and is itself coloured pale brown by the extract<sup>1</sup>. On straw the growth is similar to that of variety *pallens*, and the sporodochial conidia are again more uniform than on other substrata. Cultures on cooked potato are noteworthy; if kept at about 21° C. for 6 or 8 weeks they have a striking floral (verbena?) odour, most pronounced after exposure to air.

*Chlamydospores*. Always abundant, smooth, from cinnamon to cinnamon brown in colour; these, together with similar tinting of hyphae and conidia, cause the characteristic dark shades of cultures; position and size as in the variety *pallens* (p. 25).

*Conidia*. On all substrata conidia are much less uniform in shape and size than those of variety *pallens*, but typical conidia are of similar shape; straw bears the most uniform conidia:

(1) From sporodochia on oat straw under continuously moist conditions, temperature 10–24° C., culture 3 months old (Text-fig. 2, II): 5-septate, 96 per cent.,  $37\text{--}60 \times 4\cdot1\text{--}5\cdot2 \mu$ , average  $48\cdot6 \times 4\cdot85 \mu$ ; 6-septate, 0·5 per cent.; remainder 3- or 4-septate.

(2) From sporodochia on wheat straw slowly drying out, other conditions as in (1) above (Text-fig. 2, III): 5-septate, 96 per cent.,  $36\cdot5\text{--}52\cdot0 \times 4\cdot5\text{--}5\cdot2 \mu$ , average  $44\cdot2 \times 4\cdot97 \mu$ ; 6-septate, 3 per cent.,  $46\cdot8\text{--}52\cdot0 \times 5\cdot0\text{--}5\cdot2 \mu$ , average  $48\cdot8 \times 5\cdot15 \mu$ ; 3- and 4-septate make up the remainder.

(3) From pseudopionnotes on wheat-meal agar; cultures 8 weeks old, direct from naturally infected material: 5-septate, 99 per cent.,  $41\cdot6\text{--}65 \times 4\cdot0\text{--}5\cdot2 \mu$ , average  $50\cdot1 \times 4\cdot87 \mu$ ; 0-, 3-, 4-septate, occasional and variable. The first sub-cultures from this source show modification of conidia in the pseudopionnotes, 50 per cent. having granular contents and indistinct septation at 8 weeks old; they are of marked characteristic shape, and average  $58\cdot0 \times 5\cdot05 \mu$ . Of the 50 per cent. septate forms, 95 per cent. are 5-septate,  $39\text{--}60 \times 4\cdot7\text{--}5\cdot2 \mu$ , average  $50\cdot0 \times 5\cdot04 \mu$ ; 6-septate, 3 per cent., average  $55\cdot0 \times 5\cdot04 \mu$ .

(4) From pseudopionnotes on cooked potato, culture 90 days old, inoculum from

<sup>1</sup> See footnote on p. 25.

first sub-cultures (Text-fig. 2, III): from 50 to 75 per cent. granular, without visible septation;  $41.6-54.6 \times 4.9-5.2 \mu$ , average  $49.0 \times 5.0 \mu$ . Of the septate conidia 99 per cent. 5-septate, with reduced apical segment and dorsal curvature;  $33.8-54.6 \times 4.6-5.2 \mu$ , average  $43.3 \times 5.0 \mu$ .

(5) On saccharose, glycerine, and hard potato dextrose agar media the conidia show marked hyperbolic to parabolic curvature but are of more or less abnormal form.

*Variation in the variety nigrans.* The first monospore cultures from natural material, and (with slight differences in conidia) the sub-cultures derived from them by mass transfers, show the above-mentioned characters. When monospore cultures are made from either of the foregoing cultures a small proportion show marked modification, especially on moist, starchy media. The modified form is macroscopically similar to the variety *pallens*. It may be of vigorous growth and then develops very slowly the darker colour features of *nigrans*, or it may be non-vigorous, grow very slowly, and not develop a darker colour at all. Sometimes the growth is so restricted that the organism does not extend from the inoculum to the medium. Single-conidium growths planted as a whole on media in Petri dishes sometimes give these variants as sectoral growths. Some of the variants show extremely close resemblance to the variety *pallens*. The conidia, however, are shorter and broader, with short apical segment and reduced curvature, the contents granular, and septation indistinct. Indeed, they would not be recognised as of "gibbosum" type but for the fact that some conidia of this type are usually present also; but the greater the divergence of the variant from the normal the greater is the proportion of atypical conidia. Of monospore cultures prepared from the variants a small proportion show the usual characters of the variety, whilst the others are again variants. Sub-cultures by mass transfers from variants frequently show recovery of vigour and, though slowly, reproduction of colour features in the pseudopionnotes and media as exhibited by the normal variety. The more extreme variants, or those long cultivated, may show recovery of vigour without recovery of coloration features<sup>1</sup>. The behaviour of the variants is, therefore, decidedly erratic, even under controlled conditions; they fluctuate both in extent of divergence from type and in reaction to repeated cultivation. For this reason they are considered to be mere phenotypic changes, and not true mutants having stability under cultural conditions. When *nigrans* is recovered after passage through living

<sup>1</sup> These were thought to be transitions to the variety *pallens*, but passage of the organism through living plants proved this view to be erroneous; normal and variant forms again appeared.

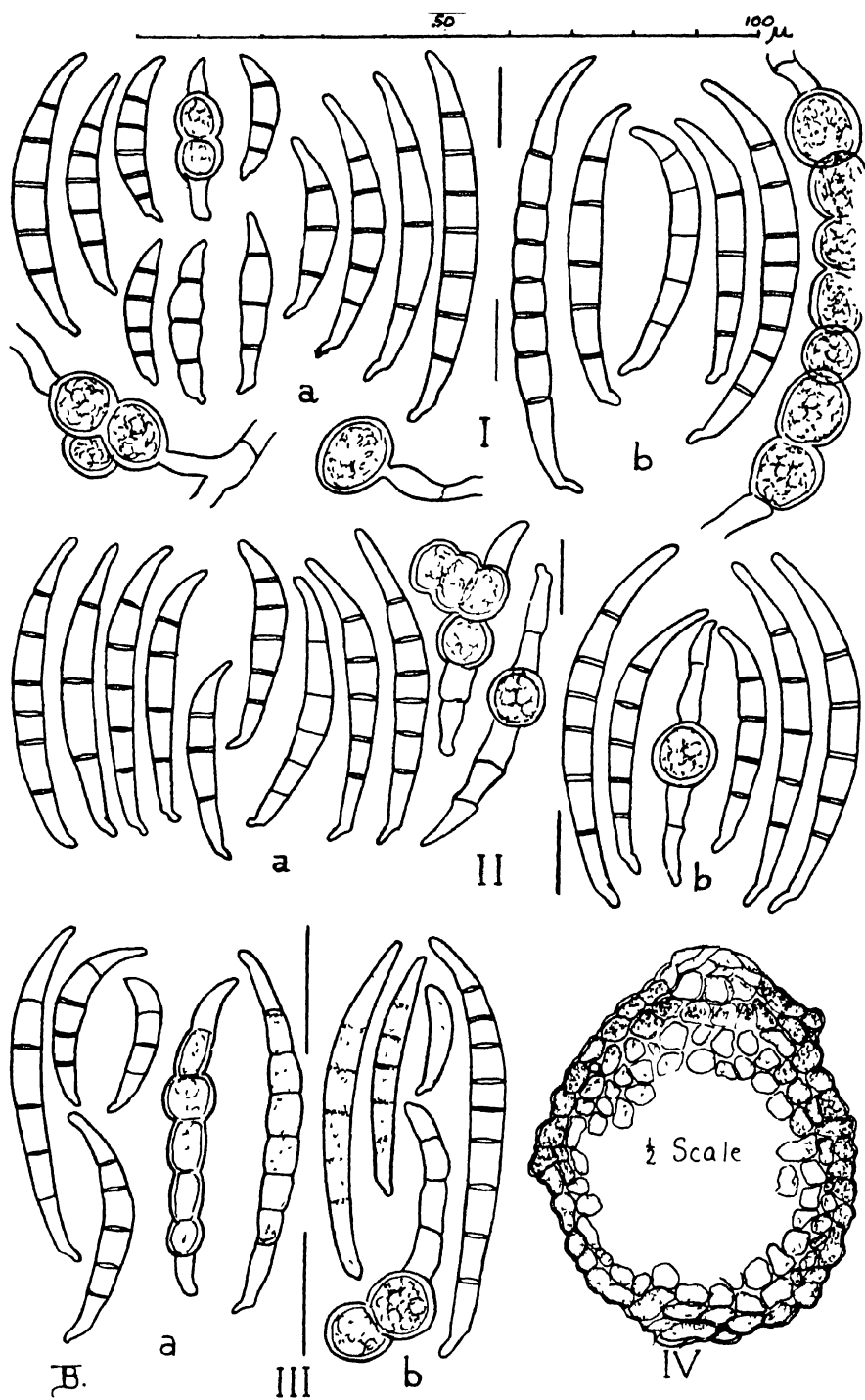
cereals (p. 31) it again appears mainly in its normal form; but in the first cultures from living material variants occur, ranging from degenerate to nearly normal forms with intermediate ones closely resembling the usual form of variety *pallens*. It is evident that the inherent capacity for variation existing in this variety is not modified by passage of the normal form through living plants, and that normal, variant, and mycelial forms are to be expected from infected natural material.

*F. Scirpi* var. *pallens* under some conditions, e.g. grown at 21° C. in good light, becomes darker in colour than usual, and then shows some resemblance to variety *nigrans*. There is, however, no fundamental modification, since the modified growth retains distinctive features such as typical conidia, relatively few chlamydospores, characteristic coloration of hard oat agar and of rice, and absence of odour on cooked potato. The change is probably correlated with the different rate of action of the metabolic processes; it is not a transition to variety *nigrans*.

#### RELATIONSHIP OF THE TWO VARIETIES OF *F. SCIRPI*.

Although a permanent change of one variety into the other has not been accomplished, the inherent instability in variety *nigrans* resulting in production of forms closely resembling variety *pallens* would indicate that the former was the progenitor of the latter. Whilst in average size the conidia of *pallens* are longer and narrower than those of *nigrans*, the latter, in older cultures in which the proportion of "gibbosum-type" conidia is increased, show an average size almost exactly the same as the former. Further, the similarity in shape and septation mode of conidia, production and form of perithecia, and general cultural characters all suggest that the two organisms are so closely related that they may rightly be considered varieties of a single species. It is fully realised that, as shown by Brown and Horne<sup>(3)</sup> for *F. fructigenum* Fries. (syn. *F. Blackmani*) and by Wollenweber for other species, variation in spore measurements and colour shades to the extent of differences between named varieties are common in sectoral variants from single-spore growths; new varieties cannot, therefore, be established on such a basis. On the other hand Leonian<sup>(4)</sup> states that a certain specific line of behaviour persists throughout all the strains and variants of a given species, the path of which may be of zigzag shape but without exceeding a specific orbit. This view correctly expresses the results of the writer's comparative studies of the varieties of *F. Scirpi*, hence the justification for distinguishing the variety *pallens* and adding the new one *nigrans* to the already extensive assemblage comprising the *Fusarium* group.





PATHOGENICITY OF *F. SCIRPI* TOWARDS CEREALS.

*F. Scirpi* has been recorded on *Triticum vulgare* in Germany and on *T. durum* in the U.S.A., the latter record being by Atanasoff who states that this fungus causes seedling blight of cereal crops(2). The present investigation has shown seedling blight to result only with seed from plants attacked in the ears (ear blight). The two varieties of *F. Scirpi* are, with one exception, of similar pathogenic capacity, and are, therefore, considered together.

Under abnormal conditions, *e.g.* with contaminated seed grown in contaminated soil in a warm greenhouse, the fungus attacks wheat and barley seedlings severely, and oats to less degree. The growing points of some of the primary and secondary roots are destroyed, and brown lesions occur at other parts; the coleoptile, especially near the seed, is affected, and conidia are frequently produced on the shoot immediately above soil level. Under these conditions most of the seedlings become more or less crippled, and some die off. Under reasonably good conditions for plant growth, *e.g.* in a cool atmosphere and no excess of soil moisture, seedlings show no such ill effects. In fact, seedlings in contaminated soil look more vigorous than the controls (Pl. II, fig. 1); this appearance is due to stimulation of early tillering in affected plants, but it is of no ultimate advantage. The plants illustrated yielded the fungus from the green and apparently healthy stems above soil level in 23 of the 28 wheat seedlings, and 23 of 29 barley seedlings in a similar trial. This unexpected result was verified by planting sound wheat in the soil which, under abnormal conditions, had caused seedling blight, immediately after removing the blighted plants; these second plants were grown under normal conditions of moisture and temperature, and all reached the stage of extruded ear, in spite of general attack on crowns and roots and sometimes casual infection on the aerial parts. It would appear, therefore,

Text-fig. 2. *F. Scirpi* var. *nigrans*.

- I. Conidia from natural materials: (a) recovered from wheat and barley seedlings grown in inoculated soil; (b) from base of a barley plant infected naturally in the field.
- II. Conidia from sterile straw, which yields a uniform type, cultures 3 months old: (a) from sporodochia on wheat straw allowed to dry out; (b) from sporodochia on oat straw kept under moist conditions; both at 10–24° C.
- III. Conidia from pseudopionnotes of second sub-cultures, less uniform: (a) from hard oat agar, culture 2 months old; (b) from cooked potato plug, culture 3 months old.
- IV. Perithecium, showing typical shape at a later stage than that of Text-fig. 1 (½ scale size).

that *F. Scirpi* is of little importance as a cause of seedling blight of cereals under ordinary field conditions.

Wheat, barley, and oats grown under approximately natural conditions show little ill effect when healthy seed is planted in contaminated soil, or when contaminated seed is planted in clean soil, and left to grow to maturity. Some reduction in vigour of plant, size of ears, and number and size of grains may be observed, indicating a slight deleterious effect on the plants during the growing season. Since there is no obvious foot rot, thinning out, or whiteheads, such reduced yield would, in practice, be ascribed to soil or season. Wheat shows the adverse effects slightly more than do barley and oats.

The ears of wheat, barley, and oats are susceptible to attack by *F. Scirpi* at any period between flowering and maturity. Under moist conditions attacks at flowering, or immediately after, check the formation or development of grain to a considerable extent; attacks during the intermediate stage of growth cause shrivelled grains, in many of which the embryo is killed; attacks on mature ears cause slight shrinkage and diffuse discoloration where the pericarp is invaded. On the glumes the usual *Fusarium* lesions are apparent, viz. pale or bleached spots with brownish margins, but they are less conspicuous than those of the more virulent species of *Fusarium*. Grains from ears inoculated before reaching maturity have, after external disinfection, yielded the fungus from as many as 100 per cent., thus showing that invasion of grains is a common result of external infection. The germination capacity of grains from such ears was reduced from 82 to 70 per cent. for wheat, and from 96 to 52 per cent. for barley, by the variety *pallens*. The reduction by the variety *nigrans* in parallel tests was insignificant—a feature closely connected with the variability of other inherent traits in the individual conidia in this variety. This is the only respect in which the two varieties have been observed to differ in pathogenicity. Affected grains that did germinate and continue growth gave weaker seedlings, with discoloured primary sheath (coleoptile) and subsequently less vigorous plants, but they did not die off during growth (Pl. II, fig. 2). The reduction in vigour and yield of plants grown to maturity is thus explained.

The pathogenic effects of *F. Scirpi* on cereals, stated briefly, comprise blight of seedlings under abnormal conditions of temperature and moisture; some reduction in vigour of growth and yield of grain when plants are attacked basally; and some check to grain formation or development, with death of embryo, following attacks on the ears by the variety *pallens*. The fungus does not appear to occur so frequently,

or to be so virulent on cereals under natural conditions as to be of serious economic importance, but it undoubtedly contributes to the damage included in the general term *Fusarium* disease, or *fusariose*, of cereal crops.

*F. Scirpi* has been recorded on *Scirpus lacustris* in France, on *Solanum tuberosum* in Germany and the U.S.A., on *Triticum durum* and *Hordeum vulgare* in the U.S.A., also on *Phaseolus vulgaris*, *Pisum sativum* and *Lupinus luteus*. To these should be added the English records on *Triticum vulgare*, *T. monococcum* and *Hordeum vulgare*.

#### SUMMARY.

1. *F. Scirpi* Lamb. et Fautr., previously described in its pseudopionotal form, occurs in a mycelial form also; the cultural characters and method for conversion of the forms are given.

2. This variety is designated *pallens* to distinguish it from a new variety designated *nigrans*, also occurring on cereals. The relationship of the two varieties is discussed.

3. Both varieties are associated with *Fusarium* disease in cereals, but are of little economic importance except when the variety *pallens* prevails on the ears of wheat and barley; infected grains used for seed, if still viable, give less vigorous plants and reduced yield of corn.

The writer tenders thanks to Dr G. H. Pethybridge for revision and criticism of this paper.

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## EXPLANATION OF PLATE II.

- Fig. 1. Wheat; healthy seed planted in soil heavily inoculated with *F. Scirpi* var. *pallens* (left) and var. *nigrans* (right); showing stimulation of early tillering and absence of seedling blight although most of the plants are infected (p. 31).
- Fig. 2. Barley grown from viable grains from ears artificially inoculated during growth and kept in a damp atmosphere for three and seven days as indicated after inoculation. *F. Scirpi* var. *pallens* (left), and var. *nigrans* (right). Grains with viable embryos continue post-germinal growth but give less vigorous plants.

(Received June 4th, 1931.)



Fig. 1



Fig. 2



THE RELATIVE RESISTANCE OF SOME WHEAT  
VARIETIES TO *TILLETIA CARIES* (DC.)  
TUL. (= *T. TRITICI* (BJERK.) WINT.)

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(1) INTRODUCTION.

THIS investigation was commenced in 1923, and in 1929 the work was submitted as a thesis for the Cambridge University degree of Doctor of Philosophy. The account that is now given is a résumé of that thesis.

This work was started to test wheat varieties for their relative susceptibility to *Tilletia caries*, but very soon it became evident that the writer was dealing not with one individual *Tilletia caries*, fixed in its specificity and individuality, but with a *Tilletia caries* that could be described as a population of individualities. In what is to follow the writer will analyse the results which he has obtained in working with this fungus, and will suggest that in the same way as a plant breeder may select from a commercial stock of a known variety of wheat a pure line for resistance to *Tilletia caries*, so may the mycologist select from a given stock of this domesticated parasite a unit from its population to which the given host is susceptible.

It will be shown also that resistance to this, the given pathogen, depends not on any one factor but on several.

Elsewhere<sup>1</sup> the literature dealing with this subject has been reviewed in detail. This being so, and for purposes of economy, no reference is made to it here.

(2) FACTORS INFLUENCING THE RELATIVE SUSCEPTIBILITY OF A  
WHEAT VARIETY TO *TILLETIA CARIES*.

(a) *Contamination experiments with a high-spore load.*

Little Joss wheat was treated with varying amounts of crushed "bunt balls," sown, and the percentage of bunted ears estimated at harvest by taking a count of 1000 ears from a diagonal band across each plot, these being six rows 16 ft. long. Table I shows this percentage from

<sup>1</sup> Dillon Weston, University Library, Cambridge: "The relative resistance of some wheat varieties to *Tilletia caries*."



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untreated and treated samples, previously contaminated at different rates with bunt balls. It is clear from these figures that infection varies directly with the spore load and that the efficiency of the dusting treatment is proportional to the rate of contamination.

Table I.

*Percentage of bunt in treated and untreated wheat when contaminated at different rates.*

Rate of treatment with bunt	Treatment	Percentage of bunted ears
1-25	Copper carbonate 3 oz. per bushel	11
	Untreated	94
1-50	Copper carbonate 3 oz. per bushel	5
	Untreated	78
1-100	Copper carbonate 3 oz. per bushel	3
	Untreated	50
1-500	Copper carbonate 3 oz. per bushel	1
	Untreated	25
1-25	Untreated	92

(b) *Contamination experiments with a low-spore load.*

In experiments carried out at two centres by the writer in co-operation with Mr C. C. Brett of the Official Seed Testing Station, Cambridge, Little Joss wheat was artificially contaminated with crushed bunt balls at rates varying from 1 part by weight of bunt to 1000 parts by weight of wheat, to 1 part by weight of bunt to 50,000 parts by weight of wheat. These results are given in Table II.

Table II.

*Percentage of bunt in untreated wheat contaminated at different rates.*

Seed contamination				Percentage of bunted ears	
				1st Trial	2nd Trial
1 part by weight of crushed bunt to 1,000 parts of wheat				4.27	6.96
"	"	3,000	"	1.44	1.21
"	"	5,000	"	0.66	0.50
"	"	10,000	"	0.41	0.29
"	"	20,000	"	0.82	—
"	"	30,000	"	0.42	—
"	"	50,000	"	1.07	0.18

Tables I and II show, therefore, that there is a direct correlation between spore load and infection. This confirms the results of other workers. This factor alone must render dubious many of the results which have been published in the past on the relative susceptibility of wheat varieties to bunt.

(c) *Date of sowing.*

In one series of experiments, wheat had been sown weekly from October, 1925, to March, 1926, each plot being six rows, a row being 16 ft. This wheat had been contaminated prior to sowing with "bunt balls" obtained from Little Joss wheat grown in 1925. It was contaminated at the rate of 1 part by weight of "bunt balls" to 25 parts by weight of wheat; from this stock the requisite amount of wheat was weighed out and sown weekly. At a later date the percentage of bunted ears was estimated by taking a count of 1000 ears in a diagonal band across the plot. Table III shows these results.

Table III.

*Percentage of bunt in Little Joss wheat sown weekly from  
October 19th, 1925, to March 29th, 1926.*

Date of sowing	Percentage of bunted ears	Date of sowing	Percentage of bunted ears
19. x. 25	68	11. i. 26	65
27. x. 25	52	19. i. 26	37
2. xi. 25	53	26. i. 26	47
9. xi. 25	32	1. ii. 26	40
16. xi. 25	42	8. ii. 26	37
23. xi. 25	60	15. ii. 26	30
30. xi. 25	72	23. ii. 26	33
7. xii. 25	58	2. iii. 26	14
14. xii. 25	61	8. iii. 26	13
21. xii. 25	61	15. iii. 26	4
28. xii. 25	64	22. iii. 26	3
4. i. 26	62	29. iii. 26	1

These figures indicate that for the variety Little Joss there is less bunt when the seed is sown in the spring. Since this experiment was carried out it has been shown to be true of all the other common English varieties. With some other wheats, however, there have been exceptions. For example, the varieties Quality and Marquillo and an unnamed Polish variety were as susceptible in the spring as they were in the winter, as were the *durum* varieties Iumello and Velvet Don. It should, however, be stated that the *durum* wheats did not stand very well the winter conditions of 1928-9. Some evidence was also obtained to show that the *spelts* were as susceptible in the spring as they were in the winter.

In addition to the above varieties numerous tests were carried out with the so-called immune and highly resistant varieties. In one experiment the variety Turkey was sown monthly from October to March. Table IV shows these results.

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Table IV.

*Percentage of bunted ears when the variety Turkey was contaminated with its own bunt and sown at monthly periods.*

Date sown	Percentage of bunted ears
October	13
November	17
December	18
January	12
February	5
March	0

Since in this experiment Turkey was contaminated with its own bunt with approximately the same spore load and seeded at the same rate under the same soil conditions, it is right to argue that this variety which is susceptible in the winter is very highly resistant in the spring.

Consider now the varieties of wheat which numerous trials have shown to be resistant. These varieties were contaminated with bunt spores and sown in the winter and spring. At harvest the bunt percentages were estimated. These figures are given in Table V.

Table V.

*Percentage of bunt in very highly resistant varieties when sown in the winter and in the spring.*

Variety	Date of sowing	Pedigree* of bunt	Percentage of bunt
Martin	24. x. 28	J. J. J. C. President	0
	11. iii. 29	"	0
Hussar	23. x. 28	J. J. J. J. Joss	0
	12. iii. 29	"	0
White Odessa	26. x. 28	J. J. J. J. Ridit	6
	14. iii. 29	"	0
Berkeley Rook	29. x. 28	J. J. J. C. Polish	2
	14. iii. 29	"	0
Sherman	31. x. 28	J. J. J. J. Joss	Less than 1
	14. iii. 29	"	0
Turkey	31. x. 28	J. J. J. C. Red Fife	8
	13. iii. 29	"	0

\* For explanation see p. 41.

In each case it will be noted that no bunt has developed in the spring. Since, at a later stage, we shall show that these resistant varieties may be susceptible, it will be necessary here to show what happens when they are contaminated with their own bunt. The results obtained from this experiment are given in Table VI.

Under these conditions, although bunt has developed, the varieties still show a very appreciable resistance when spring sown, but this

resistance is not comparable to the resistance which they show when bunted with other types of bunt spores.

Table VI.

*Percentage of bunt in very highly resistant varieties when contaminated with their own bunt and sown in the winter and in the spring.*

Variety	Date of sowing	Pedigree* of bunt	Percentage of bunt
Martin	24. x. 28	J. J. J. J. Martin	5
	12. iii. 29	"	0
Hussar	23. x. 28	J. J. J. H. Hussar	34
	12. iii. 29	"	0
White Odessa	26. x. 28	J. J. J. W. White Odessa	58
	13. iii. 29	"	21
Berkeley Rock	29. x. 28	J. J. J. Br. Berkeley Rock	71
	14. iii. 29	"	12
Sherman	30. x. 28	J. J. J. S. Sherman	33
	14. iii. 29	"	1
Turkey	31. x. 28	J. J. J. J. Turkey	50
	13. iii. 29	"	4
Ridit	22. x. 28	J. J. J. R. Ridit	31
	11. iii. 29	"	2

\* For explanation see p. 41.

(d) *Rate of seeding.*

For the past five seasons observations have been made on the effect that rate of seeding has upon the percentage of bunted ears in an infected crop. The results have indicated that the thinner the crop is seeded the less the percentage of bunted ears.

In a row of a given length there will be a greater number of ears per plot when seeded thickly than when thinly. It may be that this accounts for the difference in the infection that results.

(e) *Influence of tillage operations on the development of bunt.*

Experiments were carried out in 1925 to determine if subsoil ploughing had any effect on the development of bunt, and also to compare surface-sown seed with seed that had been drilled. The results suggested that there was little or no difference between either the subsoil ploughed and unsubsoiled plots, or the surface-sown and drilled plots.

(f) *Soil infection.*

It was thought that, in England, soil contamination might be a factor in the infection of a wheat crop. If this was so precautions would be necessary in testing wheat varieties for resistance to the disease. The following experiment was carried out.

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On October 19th, 1925, the furrows of an experimental plot were heavily contaminated with bunt spores. These were then covered with soil and left until November 23rd, when apparently clean Little Joss wheat was sown in these. At harvest there was present in these plots 8.7 per cent. of bunted ears. The control plots which consisted of apparently clean seed from the same bulk in apparently clean ground produced 1 per cent. of bunted ears. It appears, therefore, that under certain conditions contamination of the soil may be a small factor in this country in producing bunted crops. The possibility of infection arising in this manner is, however, only very slight and presumes first, a very intense attack of bunt in the original wheat crop, second a very high percentage of bunt balls shed, and thirdly wheat following wheat. It is concluded that this is a factor that need not be taken into account in England when testing wheat varieties for resistance to *Tilletia*.

### (3) THE RELATIVE RESISTANCE OF SOME WHEAT VARIETIES TO BUNT WHEN CONTAMINATED WITH (a) LITTLE JOSS BUNT, (b) THEIR OWN BUNT.

These experiments have been conducted on the University Farm, Cambridge. No standard size of plot has been adopted during the course of these investigations, but in any one series of experiments and in any one year they have been uniform in size and strictly comparable. With the exception of some plots in the 1928 trials and those in 1929 they have consisted of approximately 100 ft. of drill arranged in six rows.

In the larger plots the percentage of bunt was estimated on 1000 ears taken in a diagonal band through the plot. Where these were of small size 1000 ears, or failing that the total number, were counted.

The bunt used in these experiments was *Tilletia caries* (= *T. tritici*). Numerous microscopical examinations of bunt spores were made, but no *Tilletia laevis* was found. The bunt from every variety that was grown in 1927-8 has been examined microscopically.

The origin of the bunt used in these experiments is as follows: In 1923 a bunted sample of Little Joss wheat was obtained from the Eastern Counties Farmers' Co-operative Society. This was sown and provided the bunt for the 1924-5 experiments. Bunted Joss arising from this was used to contaminate the wheat in 1925-6, and the bunt from this was used for the next year's experiments. This strain of bunt has been preserved in this way until the present date.

Contamination unless otherwise stated was always carried out with Little Joss bunt, and this originated from the 1923 sample. No other

bunted material was obtained. If bunt was grown on any other variety and used for the purpose of contaminating other varieties this is stated and the altered pedigree is given.

The seed has been contaminated until it is literally black and has received 1 part by weight of crushed bunt balls to 25 parts by weight of wheat.

The original American varieties with which this work was started were obtained from Prof. F. L. Engledow, of the Plant Breeding Institute, Cambridge, who in turn obtained them from Dr E. F. Gaines, Washington, U.S.A. Full information with regard to these was supplied with the specimens and the Cereal Investigation number was quoted. In the course of the work other samples of the same and also different resistant varieties were obtained.

The date of sowing has varied, but in any one set of experiments it has been carried out on the same day. The rate of seeding has varied in the different experiments, but for any one series it has been the same.

In the season 1928-9 the majority of the samples were treated with formalin (1 : 320). When the seed was dry this was contaminated with the appropriate bunt spores. Previous to this it had not been treated.

In the tables which follow, the pedigree of the bunt used is shown as follows: If in 1924 the bunt used was Little Joss this is indicated by the letter J; if this was used in 1925 on Little Joss wheat the same letter is maintained; if in the next season it was grown on Sherman wheat this is indicated by the letter S. In the last season's experiments the full name is given. For example, J. J. J. R. Ridit, indicates that in 1924-5-6 Little Joss bunt was grown on Little Joss wheat, in 1927 it was transferred to the variety Ridit and in 1928 was maintained on Ridit.

(a) *The relative resistance of some resistant wheat varieties when contaminated with Little Joss bunt.*

During the past five seasons the following wheats, Sherman, White Odessa, Berkeley Rock, Hussar, Ridit and Martin, have been grown on the Cambridge University Farm and tested for their resistance to the bunt fungus. Many other wheats have been grown, but particular attention has been paid to these, since various investigators consider them immune to this disease. For this reason the major portion of this work will deal with these varieties.

The following are the results of contamination experiments over a period of years (a) in America and (b) in England. Table VII shows the percentage of stinking smut at Pullman, Washington, on these wheat

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varieties from 1919 to 1927. These figures were supplied by Dr E. F. Gaines, whose courtesy the writer here acknowledges. Table VIII shows the percentage of bunt on the same varieties when grown at Cambridge, England.

Table VII.

*Percentage of stinking smut at Pullman, Washington, on wheat varieties from 1919 to 1927.*

Variety	C.I.*	Percentage of smut								
		1927	1926	1925	1924	1923	1922	1921	1920	1919
Martin	4463	0	0	0	0	0	0	0	0	0
White Odessa	4655	0	0.6	0	0	2	0	0	9	—
Ridit	6703	0	0	0	0	0	0	0.3	—	0
Hussar	4843	0	0	0	0	0	0	0	—	—
Sherman	4430	0	0	0	0	—	—	—	—	—

C.I. = cereal investigation.

Table VIII.

*Percentage of bunt when grown at Cambridge University Farm, England. The varieties were contaminated with Little Joss bunt.*

Variety	C.I.	Years	Percentage of bunted ears
Sherman	4430	1924-5	1
		1925-6	2
		1926-7	8
		1927-8	0.8, 1.7
		1928-9	Less than 1
White Odessa	4655	1924-5	0.3
		1925-6	0
		1926-7	9
		1927-8	5.4, 2.8
		1928-9	3, 5
Ridit	6703	1924-5	0.7
		1925-6	1
		1926-7	4
		1927-8	0, 0, 0.1, 0.3, 3.1
		1928-9	Less than 1
Martin	4463	1924-5	0
		1925-6	0
		1926-7	0.5
		1927-8	0, 0.9, 0.3
		1928-9	0
Hussar	4843	1924-5	0
		1925-6	0.2
		1926-7	0.6
		1927-8	1.5
		1928-9	Less than 1
Berkeley Rock	—	1924-5	0.5
		1925-6	1
		1926-7	2
		1927-8	1.6
		1928-9	Less than 1

From the tables it will be seen that these varieties are very highly resistant to the bunt fungus. Indeed, from the American figures it would be inferred that Martin, Hussar and Sherman were immune. The English figures, however, do not indicate complete immunity but very high resistance.

If these varieties are resistant then they may be suitable for the plant breeder in the production of resistant hybrids. The case for resistance, however, must be infallible before crosses are carried out. Consider the following experiment with the variety Sherman. It was tested in 1923 at St Moro, Oregon, U.S.A., by the Cereal Investigation Board. The percentage of bunt then obtained was 1.1. In 1924-6 it was very heavily contaminated by the writer at the rate of 1 part by weight of crushed bunt balls to 25 parts by weight of wheat. At the 1925 harvest the percentage of bunt was 1.01, and in 1926 1.6. The resistance of Sherman is again shown. But, in 1926 it was resown and half of this was contaminated with Little Joss bunt and the other half with its own bunt (*i.e.* Sherman bunt that had originated in the crop of the previous year, after it had been contaminated with Little Joss bunt). At harvest in 1927 the percentage of bunt respectively in the two plots were as follows: Sherman with Little Joss bunt, 8.1 bunted ears, Sherman with Sherman bunt, 85.7 bunted ears. It is clear, therefore, that under certain conditions Sherman is not immune. Now how does this principle apply to these other resistant varieties when they are treated in the same way? They become susceptible as Table IX will show.

(b) *The relative resistance of resistant varieties when contaminated with their own bunt.*

Table IX.

*Percentage of bunted ears when certain wheat varieties were contaminated with (a) Little Joss bunt, (b) their own bunt, in the season 1927-8.*

Variety	C.I.	Seed from harvest of	Percentage of bunted ears when contaminated with	
			Little Joss	Own bunt
Sherman	4430	1927	0.8	22.2
		1926	1.7	26.8
White Odessa	4655	1926	5.4	34.7
		1927	2.8	47.6
Ridit	6703	1927	0	7.6
		1926	0.1	1
		1927	0.3	7.2
		1927	3.1	—
Hussar	4843	1927	—	19.8
		1927	0.3	14.2
		1927	1.6	91.1



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From the evidence that has been produced it is clear that under the conditions that have been described these varieties are susceptible, and it may be assumed that they are susceptible because the species of this pathogen is composed of a number of different strains. This is a logical assumption which is proved by the following experiment.

### (4) *PHYSIOLOGIC FORMS OF TILLETIA CARIES.*

Clean samples of Berkeley Rock, Hussar, Ridit and Sherman were forwarded to Mr Hans Hansen, Lyngby, Denmark, who contaminated them thoroughly with broken bunt balls from a variety grown locally. The percentage of bunt was found by taking 200 or 300 plants and counting the number of bunted *plants*. In the English tests the varieties were very heavily contaminated with broken bunt balls from the variety Little Joss. The percentage of bunt was found by taking a count of 1000 ears in a diagonal band across the plot. These results are given in Table X.

In considering the figure it should be noted that the Danish figures refer to bunted *plants* whereas the English figures refer to bunted *ears*.

Table X.

*Relative susceptibility of five American wheat varieties to T. caries when grown at Lyngby, Denmark, in 1926-7; compared with the relative resistance when grown in England during the seasons 1924-8.*

Variety	Percentage of bunted plants when grown in Denmark in 1926	Percentage of bunted ears when grown in England in the years			
		1924	1925	1926	1927
Berkeley Rock	9	0.5	1	2	1.6
Hussar	9	0	0.2	0.6	0.3
Martin	27	0	0	0.5	0.9
Ridit	0.3	0.7	1	4	0
Sherman	28	1	2	8	0.8

It is evident from the results in this table (with the exception of the variety Ridit) that those varieties which were very strongly resistant to *Tilletia caries* in England showed little or no resistance when grown in 1926 in Denmark.

It must be noted that the varieties Berkeley Rock, Hussar, Martin, Ridit and Sherman were selected by the writer to be grown in Denmark, since these had previously been tested in the United States and elsewhere and were known to be very highly resistant to the fungus. More-

over he had already grown these varieties for two seasons and considered that the claims that were made for them as regards their resistance could be substantiated. What other explanation could be adduced to account for this apparent breakdown in resistance, other than the occurrence of physiologic forms of *Tilletia caries*?

Approximately 2300 plots were laid down in the season 1928-9. The experiments to be described are selected from these, and the four varieties Little Joss, Ridit, Sherman and Hussar are discussed.

Each plot consisted of a 10 ft. run of drill. The number of the plots in a series is indicated in the tables that follow, as also are the types of bunt employed. These immune varieties were contaminated separately with Little Joss bunt and with their own bunt; in addition Little Joss wheat was also contaminated with the bunt from the resistant variety.

Tables XI, XII give the results for the variety Hussar, Table XIII for Little Joss and Tables XIV and XV the results for the varieties Sherman and Ridit respectively.

Table XI.

*Percentage of bunt in three different samples of Hussar wheat that have been grown for different periods in England. The wheat was contaminated with its own bunt and was sown on November 14th, 1928.*

(a) *Grown in England since 1924. Previously grown in Washington.*

Variety	Pedigree of bunt	No. of seeds per row	No. of tillers	Percentage of bunted ears
Hussar, C.I. 4843	J. J. J. H. Hussar	480	199	53
Hussar, C.I. 4843	J. J. J. H. Hussar	240	217	50
Hussar, C.I. 4843	J. J. J. H. Hussar	160	163	35
Hussar, C.I. 4843	J. J. J. H. Hussar	120	178	41
Hussar, C.I. 4843	J. J. J. H. Hussar	80	141	36

(b) *Grown in East Anglia since 1927. Previously grown in Wales, previous to that in Washington.*

Hussar, Ca. 609	J. J. J. H. Hussar	480	313	49
Hussar, Ca. 609	J. J. J. H. Hussar	240	241	47

(c) *Grown in East Anglia since 1927, previously in Washington.*

Hussar, C.I. 4843	J. J. J. H. Hussar	480	295	27
Hussar, C.I. 4843	J. J. J. H. Hussar	240	276	42
Hussar, C.I. 4843	J. J. J. H. Hussar	160	223	29
Hussar, C.I. 4843	J. J. J. H. Hussar	120	203	18
Hussar, C.I. 4843	J. J. J. H. Hussar	80	184	23

Table XII.

*Percentage of bunted ears in three different samples of the variety Hussar that have been grown for different periods in England. The wheat was contaminated with Little Joss bunt and was sown on November 14th, 1928.*

*(a) Grown in England since 1924, previously grown in Washington.*

Variety	Pedigree of bunt	No. of seeds per row	No. of tillers	Percentage of bunted ears
Hussar, C.I. 4843	J. J. J. J. Joss	480	204	0.5
Hussar, C.I. 4843	J. J. J. J. Joss	240	156	0
Hussar, C.I. 4843	J. J. J. J. Joss	160	138	0
Hussar, C.I. 4843	J. J. J. J. Joss	120	121	0
Hussar, C.I. 4843	J. J. J. J. Joss	80	115	0

*(b) Grown in East Anglia since 1927, previously grown in Wales, previous to that in Washington.*

Hussar, Ca. 609	J. J. J. J. Joss	480	229	0
Hussar, Ca. 609	J. J. J. J. Joss	240	207	Less than 1

*(c) Grown in East Anglia since 1927, previously grown in Washington.*

Hussar, C.I. 4843	J. J. J. J. Joss	480	212	2
Hussar, C.I. 4843	J. J. J. J. Joss	240	234	0
Hussar, C.I. 4843	J. J. J. J. Joss	160	195	2
Hussar, C.I. 4843	J. J. J. J. Joss	120	173	0
Hussar, C.I. 4843	J. J. J. J. Joss	80	180	0

Table XIII.

*Percentage of bunted ears in the variety Little Joss when it was contaminated with Hussar bunt and was sown on November 14th, 1928.*

Pedigree of bunt	No. of seeds per row	No. of tillers	Percentage of bunted ears
J. J. J. H. Hussar	480	181	47
J. J. J. H. Hussar	240	191	31
J. J. J. H. Hussar	160	183	41
J. J. J. H. Hussar	120	154	31
J. J. J. H. Hussar	80	118	40

These tables indicate that under certain conditions (i.e. with its own bunt) the variety Hussar is susceptible. There is also an indication that Hussar bunt is "less virulent" on Little Joss than is Hussar bunt on Hussar.

Table XIV.

*Percentage of bunted ears when the variety Sherman is contaminated with Sherman bunt and Little Joss bunt. It also shows the effect of Sherman bunt on Little Joss wheat. Sown on November 14th, 1928.*

Variety	Pedigree of bunt	No of seeds per row in each of six rows	Average no. of tillers per row	Average of the percentage of bunted ears per row
Sherman	J. J. J. S. Sherman	480	210	42
Sherman	J. J. J. S. Sherman	240	185	37
Sherman	J. J. J. J. Joss	480	206	Less than 1
Sherman	J. J. J. J. Joss	240	158	Less than 1
Little Joss	J. J. J. S. Sherman	480	122	20
Little Joss	J. J. J. S. Sherman	240	83	19

In this table again it is shown that Sherman is susceptible to its own bunt. There is also an indication that Sherman bunt is "less virulent" on Little Joss.

Table XV.

*Percentage of bunted ears when the variety Redit is contaminated with its own bunt and Little Joss bunt. This table also shows the effect of Redit bunt on Little Joss wheat. Sown on November 9th-10th, 1928.*

Variety	Pedigree of bunt	No. of seeds per row in each of six rows	Average no. of tillers per row	Average of the percentage of bunted ears per row
Redit c.i. 6703	J. J. J. R. Redit	480	195	15
Redit c.i. 6703	J. J. J. J. Joss	480	174	1
Little Joss	J. J. J. J. Redit	480	161	29
Little Joss	J. J. J. J. Redit	240	112	28

In the same way it is shown that Redit is susceptible to its own bunt. The table indicates also that Little Joss wheat is slightly more susceptible to Redit bunt than is Redit wheat to its own bunt.

(5) THE RELATIVE RESISTANCE OF SOME SELECTED RESISTANT  
WHEAT VARIETIES WHEN SEPARATELY CONTAMINATED WITH  
BUNT SPORES FROM DIFFERENT VARIETIES.

Numerous experiments were carried out in this section of the work, but it is not thought necessary to produce the evidence obtained from them in detail. The chief varieties tested were Sherman, Hussar, White Odessa, Berkeley Rock, Redit and Martin. Other varieties tested included Red Winter, Alaska, Marquis  $\times$  Turkey, Quality, Hope, Albit and Ora.

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The above wheats have been considered in the literature as showing resistance to bunt. In this section of the work separate samples of these varieties were contaminated with separate samples of bunt obtained from these and other wheat varieties. In this way separate samples of a particular variety would be contaminated with separate samples of bunt grown on a large number of different varieties, but all of which, presumably, were originally what we have designated as Little Joss bunt.

The results that were obtained indicated primarily that none of these wheats showed any marked resistance since they were susceptible to certain forms of bunt. It is stated here that no wheat varieties were found to be resistant to this fungus with the exception of *Triticum monococcum*—and this has been shown by other workers to be susceptible to certain forms of *Tilletia caries*. The varieties were for the greater part susceptible to their own bunt but not invariably. Martin, for example, was susceptible to bunt which had grown on White Odessa; and Red Winter to the bunt spores from Berkeley Rock and Hussar. In addition, Sherman and Hussar were susceptible to the bunt from Berkeley Rock (but Hussar was not susceptible to Sherman bunt). White Odessa was susceptible to the bunt from Hussar (but Hussar was not susceptible to the bunt from White Odessa).

It would appear, therefore, that this fungus is a pathogen consisting of physiologic forms and that, moreover, they may be separated by certain differential hosts.

### (6) THE RELATIVE RESISTANCE OF LITTLE JOSS WHEN CONTAMINATED WITH ITS OWN BUNT AND WITH BUNT SPORES FROM OTHER VARIETIES.

Up to this stage we have dealt with these so-called immune varieties only. Consider now the effect of Little Joss bunt on Little Joss wheat, and Chinese White bunt on Little Joss wheat. If the general findings in the previous part of this work are correct then we should expect to find a higher percentage of bunt when Little Joss is contaminated with Little Joss bunt than when Little Joss is contaminated with Chinese White bunt. The results of such an experiment are shown in Table XVI.

From these and other unquoted results it is considered that the repeated passage of this pathogen through the host will result in the selection of a strain of that pathogen to which the host will be susceptible.

Table XVI.

*Percentage of bunted ears when Little Joss was contaminated with Little Joss bunt and with Chinese White bunt, and was sown on November 8th–9th, 1928.*

Pedigree of bunt	No. of seeds in each of six rows	Average no. of tillers per row	Average of the percentage of bunted ears per row
J. J. J. J. Joss	480	170	71
J. J. J. J. Joss	240	156	61
J. J. J. J. Joss	160	124	46
J. J. J. J. Joss	120	118	55
J. J. J. J. Joss	80	106	51
J. J. J. J. Chinese White	480	222	43
J. J. J. J. Chinese White	240	188	39
J. J. J. J. Chinese White	160	136	41
J. J. J. J. Chinese White	120	88	39
J. J. J. J. Chinese White	80 (one row)	125	37

(7) RELATIVE RESISTANCE OF SOME DIFFERENT SPECIES OF  
WHEAT TO BUNT FROM DIFFERENT SPECIES OF WHEAT.

In the following experiments, several varieties from the different species of wheat were contaminated with bunt spores from Little Joss, American Club and Rivet. Each plot consisted of an 18 ft. row of drill and the seed was very heavily contaminated in all cases at approximately the same rate, and sown on November 14th, 1927. These results are shown in Table XVII.

Table XVII.

*Percentage of bunted ears in these varieties of various species  
in the season 1927–8.*

Variety	Percentage of bunted ears from seed contaminated with bunt spores from		
	Little Joss	American Club	Rivet
(a) <i>Triticum dicoccum</i> Schübl	37	29	30
(b) <i>T. sphaerococcum</i> Perc.	31	20	29
(c) <i>T. spelta</i> L.	1.4	2.6	2.1
Red Fife, <i>T. vulgare</i> Host.	40	25	38
(d) <i>T. durum</i> Des.	0	0	0
American Club, <i>T. compactum</i> Host.	37	63	59
Persian Black, <i>T. persicum</i> Vav.	25	19	33
Marshal Foch, <i>T. vulgare</i> Host.	46	37	—
Rivet, <i>T. turgidum</i> L.	—	25	56
(e) <i>T. polonicum</i> L.	12	12	17
(f) <i>T. monococcum</i> L.	0	0	0

In addition, further samples of *Triticum monococcum* were contaminated with bunt spores from the following wheats: Turkey, Berkeley

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Rock, White Odessa, Chinese White, Cambridge Browick, Clackamas, Masters Perfection and Sherman. At harvest no bunt was observed in these plots. In subsequent experiments we have been unable to infect this species.

It is interesting to note the percentage of bunt when American Club is contaminated with American Club bunt, and also when Rivet is contaminated with Rivet bunt. It may be, however, that no significance can be attached to these figures. From the table it appears that a *durum* variety is resistant. It must not be concluded that the *durum* group as a whole is resistant, as the next quoted experiment will show.

The following wheat varieties resistant to stem rust were received from Dr Stakman, of the Bureau of Plant Industry, Washington, D.C., U.S.A. They were tested by the writer for resistance to *Puccinia glumarum* and *Tilletia caries*. The experimental procedure was similar to the previous experiment, and the seed was sown on January 29th and 30th, 1928. At a later date Dr Gaines, of the Bureau of Cereal Investigations, U.S.A., furnished the writer with a table giving the percentages of stinking smut noted in some of these varieties at Pullman, Washington, in the period 1919-27. These figures are given in Table XVIII, and the results of the English trials in Table XIX.

Table XVIII.

*Percentage of stinking smut at Pullham, Washington, U.S.A.,  
from 1919 to 1927.*

Variety	C.I. No.	Percentage of stinking smut								
		1927	1926	1925	1924	1923	1922	1921	1920	1919
Hope	8178	0	0	—	—	—	—	—	—	—
Marquillo	6887	5	19	—	—	—	—	—	—	—
Mindum	5296	—	—	0.5	33	—	5	27	17	14
Velvet Don	2222	—	—	—	—	—	—	0	0	0
Reliance	7370	22	18	—	—	—	—	—	—	—
Marquis	3641	11	9	0	4	—	5	9	3	17
Ceree	6900	34	44	—	—	—	—	—	—	—
Progress	6902	46	64	—	—	—	—	—	—	—
Kota	5878	—	—	—	90	—	82	58	44	54

The figures in the English trials do not suggest that the *durum* and the *dicoccum* types are less susceptible to bunt than the *vulgare* types. Again, from these tables it must not be inferred that Hope is a resistant variety, since in subsequent trials with bunt of a different pedigree it has given up to 50 per cent. of bunted ears.

In the season 1927-8 forty-seven varieties of wheat from Abyssinia were tested for their resistance or susceptibility; they were contaminated separately with spores from Persian Black and Little Joss bunt balls.

There appeared to be no resistance to bunt in these varieties whether from English grown or Abyssinian grown seed.

Table XIX.

*Percentage of bunted ears in certain American wheat varieties when tested in Cambridge in 1928.*

Variety	C.I. No.	Percentage of bunted ears when contaminated with bunt from		
		Little Joss	Rivet	American Club
Hope	8178	0.5	11	2
Marquillo	6887	24	29	20
Mindum*	5296	14	21	25
Velvet Don*	2222	50	62	46
Reliance	7470	12	46	15
Marquis	3641	13	10	6
Ceres	6900	51	37	31
Progress	6902	70	76	80
Kota	5878	39	63	69
Pentad*	3322	73	70	80
Iumillo*	1736	35	22	6
Vernal Emmert†	3686	2	24	23
Webster	3780	44	37	73

\* = *Durum* species.

† = *Dicoccum* species. Remainder *vulgare*.

Many other experiments have been carried out with the different species of wheat and it has been found that in *Triticum spelta* and *T. monococcum* only, was there any marked resistance. The writer, however, places no significance on this, since he imagines that it would not be difficult to secure strains to which these species would be susceptible. Two species of wheat, *Triticum pyramidale* and *T. orientale* have not been dealt with here. They were included in certain experiments, but unfortunately did not survive the winter. It was intended to include also species of *Aegilops* in these trials, but this was not done since Dr Reichert personally informed the writer that of twenty species of *Aegilops* he succeeded in Palestine in infecting only one, *A. ventricosa*, with *Tilletia caries*.

#### (8) THE SUSCEPTIBILITY OF RYE TO *TILLETIA CARIES*.

In the season 1928-9, twenty-four separate samples of rye were contaminated with bunt from different varieties of wheat. At harvest, it was observed that in some cases bunt had appeared in the rye. The highest percentage recorded was 2.

In the season 1929-30 the experiment was duplicated, but in this case the bunt obtained on rye in the previous year was also used. At harvest traces of bunt were noticed in the rye plots both when the seed



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was contaminated with rye bunt and with bunt from certain wheat varieties. The percentage of bunt, however, was not greater when the rye was contaminated with its own bunt.

Here it is considered that rye may be useful as a host for the purpose of differentiating the different physiologic forms of *Tilletia caries* which parasitise the wheat crop.

### (9) RESISTANCE OF *HORDEUM NUDUM* AND *AVENA NUDA* TO *TILLETIA CARIES*.

Finally, experiments were carried out with the object of infecting some of the less well-known forms of barley and oats. Samples of naked barley, *Hordeum nudum*, and naked oats, *Avena nuda*, were contaminated with bunt spores from different wheats, but at harvest no infection was noted.

In concluding this study it is suggested that the wheats of the 28-chromosome number are not more resistant to *Tilletia caries* than those with the 42-chromosome number. This aspect, however, has been dealt with more fully in another paper.

It has been stated in the literature of the subject that the *durum* types have their origin in countries of the Mediterranean littoral and in Abyssinia. In their distribution northwards they proceeded only to the latitude of 57°. The *durum* types are adapted therefore to warmer environmental conditions than the *vulgare* group which originated in south-west Asia. Is it correct to assume that they will exhibit a higher susceptibility in environments more nearly corresponding to their natural environment, for example, Bulgaria, Italy and Palestine? Reichert<sup>1</sup> has expressed this opinion. This may be so, but we have noted percentages of bunt as high as 80 on a *durum* grown on the Cambridge University Farm—as an antithesis to this in another variety of *durum* we have noted none.

It is considered here that disease resistance depends not on one single factor—in this case environment—but on at least three, namely, the physiologic form of the parasite and its environment, and the “strain” of the host and its environment.

### (10) HYBRIDISATION BETWEEN “RESISTANT” AND SUSCEPTIBLE FORMS.

This work was carried out by Prof. F. L. Engledow, of the Cambridge Plant Breeding Institute. In 1925 crosses were made using supposedly bunt resistant wheats with certain familiar English ones that are known

<sup>1</sup> Reichert, I.: *Agric. Expt. Stat. Inst. Agric. and Nat. Hist. Zionest. Organ. Bull.* 9, 1928.

to be susceptible. The seed set by the  $F_1$  plants were heavily bunted with bunt from Little Joss. The  $F_2$  plants came to maturity in 1927, and every plant was carefully examined. At this stage I informed Prof. Engledow of some of my results with some of these supposedly resistant wheats. As a consequence he resolved to raise  $F_3$ 's but to bunt the seed (*i.e.* seed set  $F_2$  plants) with bunt from *both* parents and with "mixed  $F_2$  bunt" (*i.e.* from a large bunch of bunted ears from all the affected  $F_2$  plants). This was carried out save that parental bunt was unobtainable in some cases. One striking fact emerged from these experiments. Only those families for which one parental bunt was lacking for seed infection contained any  $F_3$  families entirely free from bunt.

#### (11) DISCUSSION OF WORK PRESENTED IN THESE STUDIES.

What explanation can be advanced to account for the phenomena that have been presented in these studies? Why, in the majority of cases, do such immune (*sic*) varieties become susceptible when they are contaminated with their own bunt produced on plants of these varieties, and why does the repeated passage of this pathogen through a host so often increase its "virulency"? Several deductions may be drawn and amongst these the following warrant attention. It may be assumed that the given resistant variety is not a pure line; if a pure line, it may be inferred that the selection has degenerated. By the use of this word degenerate, the writer infers adverse heritable alterations in the parent which are transmitted to the progeny. This assumption is not tenable. In considering the relationship of host to pathogen and that of pathogen to host, however, another interpretation may be placed on this ambiguous and misleading word. A temporary degeneration may result as an inability of the hosts, or hosts and pathogens, to adapt themselves to the given environment. For example, the Hungarian wheats Tisza Videki and Feherinegyei lose their "strength" when grown in England. If, therefore, the biochemical nature of the grain can be altered by different environment it may be argued that different environmental conditions will render the host, consequent on such changes, *inter alia*, more or less susceptible to infection in the seedling stage.

Another interpretation may be that this pathogen has increased in "virulency." If so it is to be inferred that the pathogen is educated to the host. But, for the parasite to be educated, either the host must degenerate, the pathogen remaining unaltered, or the host must remain unaltered and the pathogen "acquire" increased vitality. It may be argued that the host is in a state of flux, degenerating and acquiring

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increased vitality, and the same argument may be applied to the pathogen. But there is no reliable evidence that, in the true meaning of the word, the host acquires or degenerates—gains or loses—any of its fundamental characteristics. It may, however, deteriorate. This, however, is of no genetical significance.

Finally the conclusion drawn here is that *Tilletia caries* is not a single system of one identity but is composed of physiologic forms.

The repeated passage of the parasite through the host has increased the disease because this process has led to the selection of a particular form or forms well adapted to the environmental conditions.

### SUMMARY.

Some factors influencing the relative susceptibility of a wheat variety to *Tilletia caries* are recorded, and it is shown that infection varies with the spore load and with the date of sowing. The relative resistances of certain wheat varieties to bunt when contaminated separately with Little Joss bunt, their own bunt and bunt spores from other varieties are shown.

Rye is susceptible to *Tilletia caries*, but no infection is recorded on *Hordeum nudum* and *Avena nuda*.

Hybridization experiments between resistant and susceptible wheat varieties are described.

The general trend of the experiments indicates that several of the so-called immune or highly resistant varieties are susceptible when they are contaminated with bunt spores that are produced on those varieties. They indicate that the passage of the pathogen through the host increases the virulency of the disease. These phenomena are accounted for because *Tilletia caries* is not a single system of one identity but is composed of physiologic forms and the passage of the parasite through the host increases the virulency because this process leads to the selection of a particular form or forms well adapted to the environmental conditions. It is thought that resistance depends not on one single factor but on at least three, namely, the physiologic form of the parasite and its environment, and the strain of the host and its environment.

(Received April 15th, 1931.)

# STUDIES ON BACTERIA ASSOCIATED WITH THE CHOCOLATE-SPOT DISEASE OF BROAD BEANS<sup>1</sup>

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(With Plates III and IV.)

## INTRODUCTION.

THE etiology of the chocolate-spot or streak disease of broad beans (*Vicia faba* Linn.) has been in doubt. The disease occurs frequently in England, and sometimes in epidemic form causing serious losses. Paine and Lacey<sup>(3)</sup> regarded *Bacillus lathyri* Manns<sup>(2)</sup> as the pathogen, but suggested that further work on it was desirable, since doubts had been expressed as to the correctness of this diagnosis. The present writers began a study of the disease while in England during 1926. A summary of some of the work has been published (4, 5).

## ISOLATIONS FROM FIELD MATERIAL.

Through the courtesy of Dr Woodward material was secured from Oxford and Northampton, where the disease was very severe in 1926.

Isolation studies were begun on August 12th, 1926, using broad-bean pods showing typical chocolate-spot lesions. The surface of the spots was cleansed before the tissue was macerated in sterile water. Of fifteen series of dilution plates, seven were sterile, seven showed bacteria and fungi, while one showed an abundance of colonies characteristic of the broad-bean organism to be described later. Of three series of dilution plates, poured on August 16th from chocolate spots on leaves and from

<sup>1</sup> Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. The results are presented in an incomplete form because of the termination of the writers' stay in England, and because the work could not well be continued in America where the disease has not been reported. Although field studies were limited and only two strains of the organism associated with the disease were studied, still a considerable amount of experimental work was done which appears worth recording. This account of the work has been delayed because it was hoped that an opportunity might occur of continuing it; but this hope has had to be abandoned.

The work was done partly in London, through the courtesy of Profs. Brown and Paine, at the Imperial College of Science and Technology, and that of Mr Hales at the Chelsea Physic Garden; and partly in Paris, through the courtesy of Monsieur le Doctor J. Magrou, at the Institut Pasteur.

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streaks on stems, one series was sterile and the other two showed many colonies of the above-mentioned organism in almost pure culture. Three series poured on August 18th, four on August 20th and four others on August 23rd from spots or streaks on leaves, stems or pods gave in great numbers, in all cases, colonies characteristic of the same organism.

In the isolations the colonies characteristic of the organism in question were the only ones which appeared consistently and in large numbers. This fact suggested that these bacteria were more promising as the probable pathogen than other organisms occasionally found (yellow and white bacterial colonies, as well as a fungus). The other organisms, especially those with yellow colonies, when present in sufficient numbers, appeared to inhibit the development of colonies of the broad-bean organism. Transfers were made from all these types and were purified by replating for use in inoculation trials.

No further field material could be secured after August 23rd, and no further studies of the lesions in the field could be made.

### INOCULATION AND RE-ISOLATION TRIALS.

The symptoms that followed inoculations with the broad-bean organism varied somewhat according to the method of inoculation and to the environment. Inoculations through needle pricks involving vascular tissue showed a darkened area immediately about the wound, which spread rapidly under conditions of high temperature and humidity. In many cases dark streaks appeared in the green stem tissue, corresponding to the invaded vascular bundles (Plate III, D, E and G). Frequently the whole stem became involved in a comparatively short time, turned dark and collapsed (Plate III, C, D and G). Such symptoms have not commonly been reported in the field; but this is not surprising, since puncture inoculations carrying large numbers of organisms into the vascular tissues can hardly be expected to occur. Likewise, in England a combination of the conditions most favourable for the disease is not common every season over any lengthy period. High humidity and high temperature have been reported (3) to favour the development of the disease.

The symptoms that followed inoculations not involving much, if any, vascular tissue, and in which bacterial invasion occurred mainly in the subcuticular tissue and was arrested after a time by unfavourable environmental conditions, did resemble those found in the field rather closely (Plate III, I, J and M). If the lesions were but very slightly discoloured when the progress of the disease was stopped by a lowered temperature or some other unfavourable factor, the invaded region

became sunken after some days and of a chocolate-brown colour. A bronze tint sometimes appeared, due, perhaps, to the presence of air under the epidermis, whilst chocolate-brown spots on the leaves also occurred. These results suggest a causative connection between the chocolate-spot disease and the organism, but considerably more work is required with field material and under field conditions, before such a connection can be regarded as established.

The organism obtained from English material was followed through the cycle of isolation in pure culture from diseased tissue, inoculation into healthy plants, development of lesions and re-isolation in pure culture five successive times, in such a manner that the organism from the previous isolation was employed for inoculation (Table I).

Similarly, another strain of the organism, secured in Paris, from a lesion that developed spontaneously on one of the stock plants being grown for experimental purposes, was followed five times through the same cycle (Table I).

Table I.

*Summary of successive isolation and inoculation studies of the broad-bean organism on broad beans, August 20th, 1926, to April 14th, 1927.*

Source of diseased material	Date of isolation of culture used	Date of inoculation	Beans inoculated with broad-bean organism		Beans punctured but not inoculated	
			Inoculated No.	In-fected No.	Punctured No.	In-fected No.
Natural infection	Aug. 20	Sept. 3	16	16	32	0
Inoc. of Sept. 3	Sept. 14	Sept. 22	8	7	4	0
Inoc. of Sept. 22	Sept. 30	Oct. 7	8	8	6	0
Inoc. of Oct. 7	Oct. 10	Oct. 14	3	3	3	0
Inoc. of Oct. 14	Oct. 18	Nov. 5	6	6	6	0
Natural infection	Feb. 26	March 3	6	6	6	0
Inoc. of March 3	March 5	March 8	6	6	6	0
Inoc. of March 8	March 10	March 14	4	4	4	0
Inoc. of March 14	March 17	April 7	8	8	8	0
Inoc. of April 7	April 8	April 14	5	5	5	0

Parallel to the successful inoculations with the broad-bean organism, repeated attempts were made to produce infection with the other organisms that appeared from time to time during the original isolation studies, viz. the bacteria with white and yellow colonies and the fungus. Likewise a number of inoculations were made with a museum culture *B. lathyri*. In all cases the results were negative.

In addition to inoculation and isolation trials with broad beans the organism was tested against garden peas (*Pisum sativum* Linn.). In one

series of inoculations with fifty garden peas forty-eight of the inoculated plants became diseased, while all twelve of the control plants remained healthy. The elongated stem lesions following inoculation appeared water-soaked, then sunken and later somewhat discoloured. They corresponded rather closely to those reported by Cayley (1), and suggested the existence of a relation between the broad-bean organism and *Pseudomonas seminum* Cayley (1). A repetition of these inoculations on peas gave similar results.

The environmental conditions favourable to infection appeared to be comparatively exacting. Although no close determinations of these conditions could be made, best results were secured between 20° and 30° C. and in conditions of high moisture both within and around the plants. Under conditions of lower temperature and humidity inoculations were unsuccessful, and well-developed lesions were prevented from making further progress. In this connection it is interesting that chocolate spot of broad beans has been reported (3) as most serious in warm and wet seasons.

#### HISTOLOGICAL STUDIES.

Material from the region of incipient attack as well as from the older lesions was fixed (chiefly in formol-acetic-acid-alcohol), dehydrated, infiltrated, embedded in paraffin and sectioned. Flemming's triple stain was commonly employed, with the modification that the slide was treated with Lugol's iodine solution immediately after the aqueous solution of gentian violet. In this way the Gram-positive bacteria were given the Gram stain within the tissue. Under the microscope they appeared in sharp contrast as purple rods against a red or orange background.

The bacteria were found in parts of two general regions in a newly invaded portion of stem. In the vascular elements they appeared first within certain vessels which occasionally seemed to be entirely filled. When the invasion was a little more severe, they occurred also in the intercellular spaces of the tissue about the vessels, and subsequently inside these cells. This tissue later collapsed into a disorganised mass (Plate IV, A) in which the bacteria usually appeared, whilst blackening of the invaded tissue progressed rapidly. Macroscopically blackened streaks showing the positions of invaded vascular bundles were frequently seen following deep puncture inoculations (Plate III, D, E and G). These became much more conspicuous after the green stems had been bleached in alcohol.

In the subcuticular region of the stem the bacteria appeared first in the intercellular spaces (Plate IV, B) and subsequently within the cells.

The tissue then collapsed, and in the later stages appeared quite disorganised. The amount of blackening of the tissue depended upon the extent of the bacterial invasion. When the invasion was arrested by unfavourable environmental conditions, the lesion appeared merely as a brown streak (Plate III, I and J), a type of lesion that might perhaps be expected under natural conditions.

#### BACTERIOLOGICAL STUDIES.

Bacteriological studies were made on two strains of the organism pathogenic to broad beans. These strains had been passed five successive times through the cycle of isolation, inoculation, infection and re-isolation. They appeared practically identical in all the characters studied.

The purity of the cultures employed was not seriously questioned. The rapidity with which the bacteria invaded the host tissue and the five successive inoculations and isolations made it seem unlikely that secondary organisms might be mixed in without showing some evidence of their presence. However, the strains were replated four successive times from 18-hour-old liquid cultures. The successive dilutions were made from broth to broth, before mixing with agar, as an added precaution to secure a satisfactory distribution of the organisms. The colonies selected were taken from plates in which less than fifty colonies were present. In each case the colony chosen was examined under the microscope to decrease the possibility of mixture of two colonies. The pathogenicity of the cultures used for bacteriological determinations was checked from time to time by further successful inoculations into broad beans.

The methods employed in making the determinations, unless otherwise indicated, were those given by the Committee on Bacteriological Technique of the Society of American Bacteriologists<sup>(6)</sup>. The tests were ordinarily run in triplicate for each of the strains, with the customary controls. They were repeated, unless otherwise noted, either two or three times.

#### *Morphology.*

The organism was a short rod with rounded ends usually occurring singly. In size it corresponded very closely with *Ps. seminum*<sup>(1)</sup>.

Attempts to prove the existence of endospores gave negative results. In material from old cultures stained by Dorner's method no spores were found. At the same time oval bodies resembling those described by Cayley<sup>(1)</sup> were observed. However, cultures containing such oval bodies



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were killed by heat at 60° C. for 10 minutes. Consequently it is considered that these oval bodies are not endospores.

Capsules were demonstrated by the flagella stain, but efforts to stain them by Borrel's method and the Chinese-ink method were unsuccessful. The cultures seemed to produce a considerable amount of slime that was easily detached from the organisms and showed as a mucilaginous background on the stained smears.

The organisms were actively motile. In hanging drops they have been seen to move past one another quite vigorously. Loeffler's method showed peritrichiate flagellae, the number varying considerably but usually being from ten to fourteen.

The bacteria were Gram-positive. This character was found very useful in separating the broad-bean organism from contaminating Gram-negative organisms. It was valuable also in demonstrating the bacteria within the host tissues. The organisms were not acid-fast.

### *Cultural characteristics.*

The cultural characteristics of the two organisms were studied on a series of different media. Unless otherwise noted, the incubation temperature was approximately 22° C.

The colony characters on nutrient agar after 2 days were as follows: growth, rapid; form, circular; surface, smooth; elevation, raised; edge, entire; internal structure, finely granular. After three or more days the surface became radiately ridged (Plate III, L). In some cases where there was abundant humidity the colonies showed a rhizoid form. This was more conspicuous in glucose nutrient agar (Plate III, K). Lens-shaped, deep colonies and thin flat colonies against the glass were conspicuous in plate cultures.

On fresh nutrient agar slants the cultures at the end of the first day were either spreading or rhizoid on the lower part, and filiform on the upper. On the second day the upper part of the slant showed the following characters: growth, abundant; form, echinulate; elevation, raised; lustre, slightly dull; optical character, opaque; chromogenesis, white to cream; odor, none; consistency, butyrous to slightly membranous; medium, unchanged. The lower end of the slant appeared contoured. With greater age the lustre became somewhat more dull, and more of the surface became contoured.

On gelatin stab cultures after 3 days, liquefaction was crateriform. After 2 weeks it was stratiform and had extended down 12 to 15 mm. A thick pellicle and a considerable flocculent precipitate were noticeable

in the liquefied portion. No change was produced in either the colour or the odour of the medium.

In nutrient broth a pellicle was formed after 5 days. The solution was slightly turbid. The moderate amount of sediment appeared viscid on agitation. There was no odour.

On potato plugs growth after 2 days was spreading and coloured cream to yellow. After 3 days the yellow colour was slightly intensified, the surface was somewhat dull and wrinkled. This cream to yellow colour was fairly common in media containing carbohydrates.

In litmus milk, after 2 weeks, the inoculated tubes had changed in colour toward buff. A similar change, which is not uncommon among bacterial cultures, was reported for *Ps. seminum*(1).

The fermentation of various sugars and related substances was studied in a limited way. Nutrient agar slants were employed to which litmus and sterilised carbohydrate solutions were added under aseptic conditions after the medium was sterilised. The sugars and related substances were added to make a concentration of 1 per cent. No gas was formed in any case. Acid was formed in most cases in 2 days, but final results were taken at the end of 9 days. Acid was formed from arabinose, dextrose, levulose, sucrose, and glycerine. No acid was formed from lactose.

No diastatic action was observed. The tests involved the use of streak cultures on Petri dishes containing soluble rice starch in nutrient agar. After 2 and 4 days respectively, different sets of dishes were flooded with iodine. No clear zone was observed.

No indol was found with the method of Salkowski. Cultures in peptone broth, incubated for 7 days, were employed.

Neither nitrite nor ammonia was produced from nitrate. The bacteria had been grown on artificial media several months when this test was made.

The oxygen requirements were examined in several ways. Transfers were made to melted nutrient sucrose agar and the organisms were uniformly distributed before the agar solidified. Abundant growth occurred on the surface, and some growth appeared throughout the deeper portions of the agar. As a variation, agar was melted and bacteria were transferred as before. However, while the medium was still liquid, it was drawn into long capillary tubes. These were sealed at both ends in a flame. Growth in small amounts occurred throughout the medium in these capillary tubes. Further tests were made with cultures on agar slants from which the oxygen was absorbed with pyrogalllic acid and potassium hydroxide. After 3 days such tubes showed a small amount of

growth. Similar tubes from which oxygen was not absorbed showed abundant growth. These tests showed that the organisms were able to produce some growth without free oxygen.

A non-pathogenic strain of bacteria was secured in some of the early isolation plates, and, since its growth characters were very similar to those of the pathogenic strains, it was carried in parallel tests with them through all the various bacteriological studies. Except for minor differences in degree the bacteriological characters were the same. Repeated puncture inoculations with this strain showed no difference from the checks. Parallel inoculations with the pathogenic organisms showed successful infection, so that the host plants and environmental conditions were satisfactory.

#### DISCUSSION.

The identity of the broad-bean organism here described is a matter of some doubt. The writers have not worked with a sufficiently large number of strains to feel that its characterisation is fully satisfactory. However, from what has been done an interesting correspondence with the description given for *Ps. seminum* (1) has become evident.

The chief differences between the two organisms may here be considered briefly. *Ps. seminum* has polar while the broad-bean organism has peritrichiate flagellae. Variation in strains of the organism or in staining methods might be considered in connection with this difference. Presence and absence of endospores can hardly be considered a difference, since bodies corresponding to the "endospores" of *Ps. seminum* were observed in cultures of the broad-bean organism. The use of the term "endospores" for such bodies is open to question. A clear-cut difference appears in the fact that acid is formed in culture media containing glycerine by the broad-bean organism, and not by *Ps. seminum*.

Comparison of the characters of the broad-bean organism with those reported (2, 3) for *B. lathyri*, however, shows that these two organisms differ in a number of characters.

Although the information available concerning this broad-bean organism certainly places it close to that described by Cayley (1), yet its exact status in relation both to *Ps. seminum* and to chocolate spot or streak of broad beans remains to be more fully determined as a result of further investigations.

## SUMMARY.

A bacterium which resembles *Ps. seminum* Cayley has been isolated from broad beans showing symptoms of chocolate spot in the field. The broad-bean organism was found to be vigorously pathogenic to broad beans and to garden peas grown in the greenhouse. Two strains have both been passed five successive times through the cycle of isolation, inoculation into broad-bean plants, the production of disease, and re-isolation. Further study is necessary before a causal connection between this organism and the disease can be regarded as established.

Temperatures between 20 and 30° C. and high humidity favour infection.

Histological studies of stems which became diseased following puncture inoculations showed the presence of the broad-bean organism within the vessels, in the intercellular spaces, and inside the cells of the invaded tissue. The tissues about the vessels and immediately under the epidermis seem to be invaded earlier than other tissues.

Bacteriological characters of the broad-bean organism are described.

A non-pathogenic strain was found which corresponded in bacteriological characters with the pathogenic strains.

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# EXPLANATION OF PLATES III, IV.

## PLATE III.

The results of control punctures and puncture inoculations with the broad-bean organism on peas and broad beans, and the colony characters of this organism on agar plates.

- A. Control puncture on a garden-pea plant in a series with B.
- B. Result after 4 days from a puncture inoculation.
- C, D and E. Results after 3 days from puncture inoculations on broad-bean stems. In D, E and G dark lines of invaded tissue appear at *s*.
- F. Control punctures in a series with C, D and E.
- G. See C, D and E.
- I and J. Chocolate brown sunken streaks produced in the outer cortex of broad-bean stems as they appeared 2 weeks after inoculation. The invasion in these cases was arrested by a lower temperature after the infection had become well started. A still less advanced case appears at M. A control puncture appears at N.
- K. Growth of the broad-bean organism on glucose nutrient agar with abundant moisture after 4 days at 20° C. The surface colonies are rhizoid. The deeper colonies are lenticular. Thin flat colonies appear against the glass.
- L. Growth of the broad-bean organism on nutrient agar but with less moisture than in K, after 4 days at 20° C. The surface colonies appear rugose. The deeper colonies and those against the glass appear as in K. (Slightly magnified.)
- M and N. See I and J.

## PLATE IV.

Photomicrographs of broad-bean stem tissue invaded by the broad-bean organism.

- A. Cross-section showing the collapse of certain tissues, especially those about the vessels and beneath the epidermis, after the invasion of the broad-bean organisms. Groups of bacteria appear as dark spots in the invaded tissue.
- B. Cross-section showing the bacteria as black spots mostly in the intercellular spaces of partially collapsed subepidermal tissue shortly after invasion by the broad-bean organisms.

(Received June 4th, 1931.)



RIKER AND RIKER - STUDIES ON BACTERIA ASSOCIATED WITH THE CHOCOLATE SPOT DISEASE OF BROAD BEANS (pp. 55-64).





RIKER AND RIKER —STUDIES ON BACTERIA ASSOCIATED WITH THE CHOCOLATE SPOT DISEASE OF BROAD BEANS (pp 55-64).





# THE GROWTH AND RESPIRATION OF BACTERIA IN SAND CULTURES IN THE PRESENCE AND ABSENCE OF PROTOZOA<sup>1</sup>

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(With 8 Text-figures.)

## PART I.

THE results of Cutler, Crump and Sandon<sup>(1)</sup> and of the author in Hungary<sup>(14)</sup>, on the counting of protozoa and bacteria in the soil have shown that an inverse correlation exists between their numbers. This result has led to the conclusion that protozoa have the effect of reducing the number of bacteria by their phagocytic action not only under the special circumstances described by Russell and Hutchinson<sup>(10, 11)</sup>, but also under ordinary field conditions.

On the other hand very little information is available as to the other activities of protozoa in soil, though more recent work has given some very unexpected results. Nasir's<sup>(9)</sup> experiments showed that the presence of protozoa in artificial culture media or in sand cultures had no depressing effect on nitrogen fixation, but that on the contrary it caused a great increase in the amount of nitrogen fixed. In the year 1926 the same result was confirmed in different parts of the world. Cutler and Bal<sup>(2)</sup> in England suggested that the increased nitrogen fixation might be due to the efficiency of *Azotobacter* being maintained for a longer period; Keizo Hirai and Iwao Hino in Japan<sup>(7)</sup> also found that nitrogen fixation was generally stimulated in the presence of protozoa. In their opinion the soil protozoa and bacteria live in a state of disjunctive symbiosis, in other words the presence of soil protozoa decreases the acidity of the nutrient medium, which results in a vigorous growth and increased nitrogen fixation. The present writer in Hungary<sup>(13)</sup>, at the same time and without previous knowledge of these results, published the results of an investigation on the influence of various artificial zeolites on nitrogen fixation by pure and mixed *Azotobacter* cultures. It was found that the nitrogen-fixing power of the new cultures, especially those infected by

<sup>1</sup> This work was carried out at Rothamsted whilst the writer held a post-graduate Fellowship of the Royal Hungarian Ministry of Education.

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protozoa (*Paramoecium* sp. and *Colpidium* sp.) in the presence of readily available salts, was greater than that of pure cultures; this effect being probably due to the stimulating effect of the protozoa. In 1928 Fedorowa-Winogradowa(4) carried out experiments cultivating *Azotobacter* and soil amoebae together on sterile soil and stated that, while the amoebae reduced the number of *Azotobacter*, at the same time they stimulated their rate of reproduction. In sterile medium consisting of 1 per cent. mannitol solution and soil the development of *Azotobacter* was more vigorous in the presence than in the absence of amoebae.

The protozoa have also a decidedly beneficial effect on other common soil processes. Hills(6) studied the accumulation of available nitrogen ( $\text{NH}_3$  and  $\text{NO}_3$ ) in sterilised soil; on the one hand reinoculated with soil known to contain protozoa, and on the other with a crude culture of bacteria obtained by picking several colonies from agar plates which had been poured from soil dilutions. He obtained no evidence of decreased accumulation of available nitrogen in the presence of protozoa. Waksman(15) found that the protozoa seemed to have a detrimental effect upon the numbers of bacteria but not upon their ammonifying efficiency. Skinner(12) partially sterilised soil, and after reinoculating with various cultures he found that *Hartmanella hyalina* caused a reduction in the number of bacteria and a slight depression in carbon dioxide evolution and ammonia accumulation; the fungi *Trichoderma Kőningi* and *Penicillium*, however, caused an increase in carbon dioxide evolution and a decrease in ammonia accumulation. Neither of these workers attempted to follow the course of ammonia formation by consecutive observations. Meiklejohn(8) carried out two sets of experiments on ammonia production from peptone, one with bacteria in liquid cultures and another in which bacteria and *Hartmanella* were compared with bacteria alone in sand cultures. The presence of amoebae, while lowering the bacterial numbers, appeared to increase the rate of ammonia production, and it is suggested that the amoebae reduced the bacterial numbers from too high a value to a value nearer the optimum for ammonia production, and so increased the rate of ammonia production.

With regard to the carbon dioxide production from soil it is well known that this is probably a better index of soil fertility than the actual number of soil bacteria; though many investigators have found a close connection between carbon dioxide evolution, numbers of soil bacteria and soil fertility. According to the experiments of Cutler and Crump(3) on carbon dioxide evolution from soil and sand cultures containing a species of bacterium with and without amoebae, however, the

correlation would appear to be not such a simple one as that inferred from the previous work, since bacterial numbers and carbon dioxide production are correlated only provided that the amoebae are not present, or are present in very small numbers. In sands containing peptone, the amoebae caused a decrease in carbon dioxide production, but in sands containing mineral salt solution and glucose or soil extract the reverse effect was obtained.

From the above results it seems to be well established that soil protozoa may not only act as harmful factors in the life of soil bacteria, but they may also stimulate bacterial development, resulting in further biological transformations in soil. This fact may be connected with their phagocytic action, but up to the present little work has been done in this direction. It was felt that the importance of this problem in soil processes was such as to warrant further investigations on the influence of protozoa on carbon dioxide production. The problem involves so many factors and is one of such great complexity, that it was necessary to simplify it. This was effected by studying the influence of protozoa (especially Ciliates and Flagellates) on the carbon dioxide production of mixed and pure bacteria cultures in sand containing definite compounds.

#### METHODS.

Although soil would undoubtedly appear to be the best medium to use, preliminary experiments showed that the results were difficult of interpretation owing to its complex nature. The problem was therefore simplified by using sand. Silver sand, which passed a 1 mm. sieve, was digested with strong hydrochloric acid for 24 hours, washed free of acid in running water, dried and ignited. Four hundred gm. portions of this were then placed in 2 litre Erlenmeyer flasks and sterilised in an autoclave under 15 lb. pressure for 30 min. The moisture contents were made up by adding the following two nutrient solutions: (1) mineral salt solution + 0.5 per cent. peptone; (2) mineral salt solution + ammonium sulphate + 0.6 per cent. glucose (ratio C/N = 3.5 : 1) in amount equivalent to 16 per cent. of the weight of the dry sand.

The method of inoculation, if not stated otherwise, was as follows. Four different cultures were employed: (a) protozoa<sup>1</sup> + bacteria from Barnfield farmyard manure plot; (b) mixed soil bacteria from soil dilutions enriched by several sub-cultures in peptone, or ammonium

<sup>1</sup> The protozoa consisted of *Oicomonas termo*, *Cercomonas crassicauda*, *Heteromita* sp. and other Flagellates.

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sulphate media; (c) pure cultures of "YB" bacteria either without *Colpidium* sp. or (d) with the addition of *Colpidium* sp. The sand after thoroughly mixing with the nutrient media was inoculated under aseptic conditions by spraying it with 10 cm. of the cultures. On account of the sticky nature of the peptone the sand was stirred every day throughout the experiment. This was done aseptically for both series of cultures. Bacteria and protozoa were counted daily by the dilution method used in this laboratory. Later, in order to facilitate the work, only bacterial counts were made daily, but microscopic examination of a small quantity of sand in a drop of sterile water on the slide was carried out every day to ensure that the protozoa were growing satisfactorily.

For the determination of the carbon dioxide production the Pettenkoffer method was employed, using 0.2 per cent. baryta solution for the absorption and  $N/5$  hydrochloric acid for titration with phenolphthalein. In every case the cultures were aerated by drawing carbon dioxide free air over them 10 hours daily by means of an aspirator. Before each experiment was begun the apparatus was made free of carbon dioxide by  $\frac{1}{2}$  hour aeration with carbon dioxide free air. The baryta solution was titrated at least once in every 24 hours or oftener if necessary. All the experiments were repeated three or four times under quite uniform conditions.

### RESULTS.

In the first experiments with peptone the carbon dioxide evolution began slowly and reached its maximum in the second or third day in the majority of cases and then fell, first sharply and later more steadily. During this time the growth of bacteria followed quite closely the evolution of the carbon dioxide, and as found by Cutler and Crump(3), it reached its maximum a day later. This occurred in all the experiments made on peptone media inoculated either with bacteria only or with bacteria and protozoa. No explanation for this interesting observation has yet been found. In other respects, however, there were great differences according to the inoculations, since the cultures containing protozoa produced always much larger amounts of carbon dioxide than those with bacteria alone, and the mixed soil bacteria also produced more carbon dioxide than the pure "YB" culture. Typical curves showing the carbon dioxide production during the experiments and the differences between the amounts given off in various cultures are given in Fig. 1, calculated in mg. carbon dioxide per gm. of sand.

Another picture is obtained in the experiments with ammonium sulphate and glucose. In this case a C/N ratio of 3.5 was chosen in order

to correspond with the C/N ratio of the peptone solution. In these experiments the connection between the bacterial growth and carbon

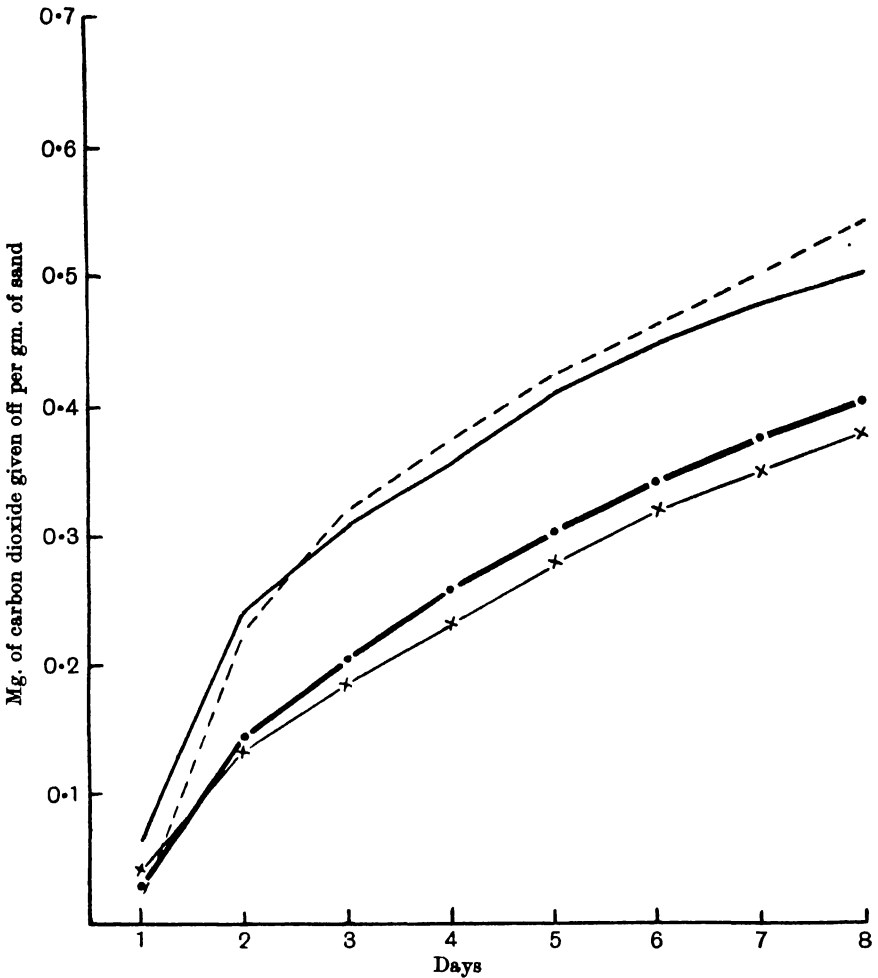


Fig. 1. Influence of protozoa on the  $\text{CO}_2$  production. Peptone.

- Soil bacteria and protozoa, mixed culture.
- "YB" bacteria and *Colpidium* sp.
- Mixed soil bacteria.
- ×——× "YB" bacteria.

dioxide production was not so close as before, though a similar lag of the maximum bacterial number behind the maximum of carbon dioxide production could be observed in the majority of cases. This experiment was characterised by considerable fluctuations in bacterial numbers

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which contrasted with the uniformity observed in the peptone experiment.

To study the question whether a close relation existed between the number of bacteria and carbon dioxide produced, contingency tables were drawn up in which the two variables were the carbon dioxide production and the numbers of bacteria. An increase in either variant is shown by a + sign and a decrease by a - sign, if both increase or decrease together it will be shown by ++ or --, if they vary inversely by +- or -+. If there are a sufficient number of cases, and the variables are wholly independent, there will be equality between the like and unlike signs; a preponderance of like or unlike signs will show that the two variables are related one to another. To test the significance of any departure from equality a  $\chi^2$  was worked out: if the  $\chi^2$  is greater than 4 it may be assumed that there exists a relation between the variables which is not due to chance.

In Table I it is shown that the relationship was very close between the bacterial numbers and carbon dioxide produced in the peptone experiments, not only when bacteria were present alone, but also in the presence of protozoa. In the medium containing glucose + ammonium sulphate the value of the test of relationship is hardly significant, especially in the presence of protozoa. It is interesting to note that the number of bacteria on peptone media often reached several thousand millions, while in the glucose + ammonium sulphate media greater numbers than 400 millions per gm. were not observed. In view of the lower bacterial numbers on the glucose medium it was thought desirable to determine to what extent the carbon dioxide production could be accounted for by the fungal growth or the presence of bacteria not estimated by Thornton's medium.

Table I.

*Contingency tables for carbon dioxide production and bacterial numbers in sand (CO<sub>2</sub> given first).*

Sands		++	+-	-+	--	$\chi^2$
Peptone	Bacteria alone	11	0	4	17	19.00
	Bacteria and protozoa	8	0	4	20	17.77
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	Bacteria alone	9	2	4	9	5.97
	Bacteria and protozoa	10	4	3	7	4.03

With this object in view plate counts were made on Czapek agar for fungi and on glucose + ammonium sulphate agar for bacteria. It was found that there was no significant difference between the two sets of

estimations. It must therefore be concluded that no special bacteria or fungi were responsible for the carbon dioxide production obtained.

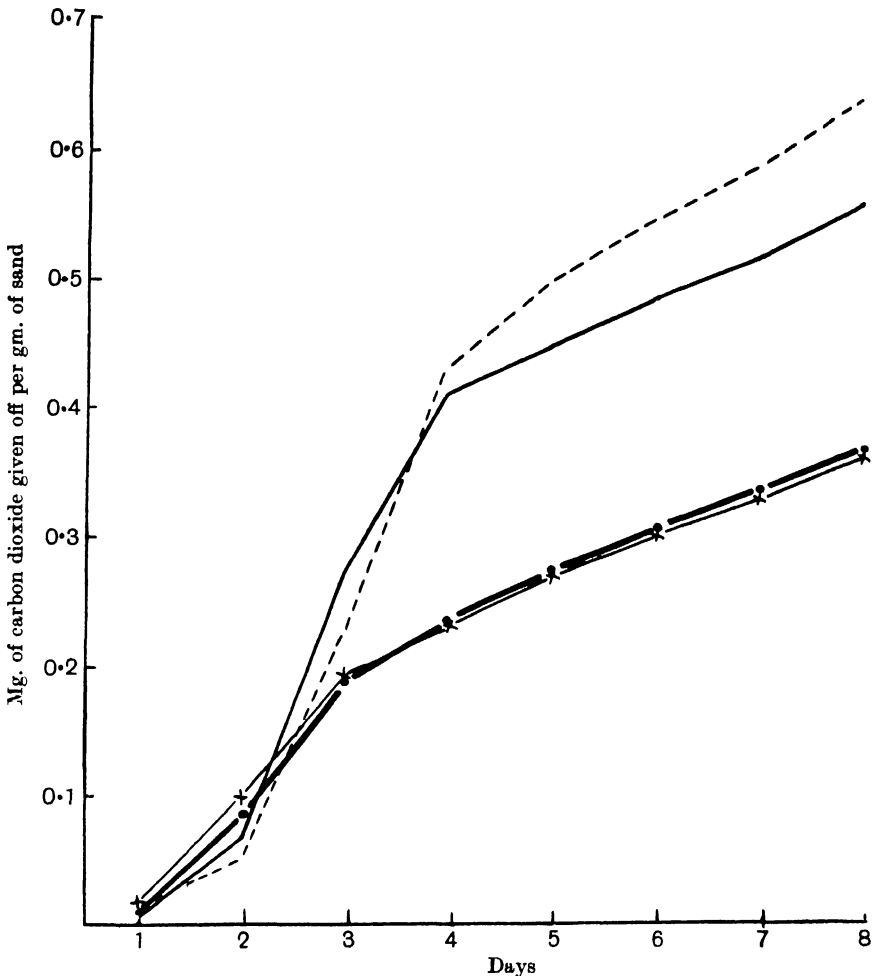


Fig. 2. Influence of protozoa on the  $\text{CO}_2$  production.  $(\text{NH}_4)_2\text{SO}_4$  + glucose.

- Soil bacteria and protozoa, mixed culture.
- "YB" bacteria and *Colpidium* sp.
- —● Mixed soil bacteria.
- × —× "YB" bacteria.

The results of the experiments made on glucose + ammonium sulphate media are shown in Fig. 2. It is interesting to note that the protozoa-free cultures gave off nearly the same amount of carbon dioxide. The cultures infected with protozoa showed an increase in the carbon



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dioxide evolution and this increase was especially great in the case of the culture obtained from soil and containing bacteria and protozoa.

The bacterial efficiencies are given in Table II according to the number of bacteria present per gm. of soil. For the measurement of this efficiency the amount of carbon dioxide produced during each 24-hour period was calculated per 1000 million bacteria.

Table II.

*Bacterial efficiencies in gm. per 1000 million bacteria.*

Sands	No. of cases	0-200 millions	No. of cases	200-400 millions	No. of cases	400-600 millions	No. of cases	600-800 millions	No. of cases	Over 800 millions
Peptone										
Bacteria alone	5	0-000612	5	0-000155	3	0-000083	2	0-000149	6	0-000082
Peptone										
Bacteria and protozoa	6	0-000396	6	0-000240	4	0-000277	2	0-000123	2	0-000144
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>										
Bacteria alone	12	0-000565	9	0-000249	—	—	—	—	—	—
Bacteria and protozoa	15	0-000691	6	0-000524	—	—	—	—	—	—

It is obvious from this table that the bacteria alone on both media show very varying efficiency. In the presence of protozoa, however, the differences between the efficiencies of various bacterial populations were not so large. This fact is of interest, because it indicates that the protozoa exert a control on the bacteria, or in other words they have an equalising effect on the work done by the bacteria. The bacterial numbers in pure cultures were greater in every case than in the cultures with protozoa, especially at the time of maximum carbon dioxide production; but nevertheless not only was the efficiency more uniform, but the production of carbon dioxide was also higher in the latter cases; as is shown in Table III.

Table III.

*Amount of CO<sub>2</sub> in gm. given off from 400 gm. of medium  
by varying numbers of bacteria.*

Sands	No. of cases	0-200 millions	No. of cases	200-400 millions	No. of cases	400-600 millions	No. of cases	600-800 millions	No. of cases	Over 800 millions
Peptone										
Bacteria alone	5	0-0103	5	0-0139	3	0-0174	2	0-0412	6	0-0333
Bacteria and protozoa	6	0-0144	6	0-0291	4	0-0539	2	0-0333	2	0-0501
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>										
Bacteria alone	12	0-0111	9	0-0298	—	—	—	—	—	—
Bacteria and protozoa	15	0-0215	6	0-0585	—	—	—	—	—	—

According to these data the effect of the protozoa is to reduce the bacterial numbers and at the same time to maintain their efficiency on the same level. In this connection it should be observed that Cutler and Crump (3) also found that the bacteria produce more carbon dioxide when their numbers are not rising and less as their numbers increase. The corresponding data obtained in the present investigation are given in the contingency Table IV, in which the two variables are the bacterial numbers and their efficiency.

Table IV.

*Contingency table for numbers of bacteria and efficiency in producing carbon dioxide (efficiency given first).*

Sand	Whole period				$\chi^2$
	+ +	+ -	- +	- -	
Peptone	6	23	22	5	20.6
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	8	14	16	4	8.1
Both media	14	37	38	9	28.1

It is seen that in most cases increasing efficiency is connected with decreasing bacterial numbers, the converse also being true. The significance of this fact becomes more obvious if only the last 5 days are taken in consideration (Table V).

Table V.

*Contingency table for numbers of bacteria and efficiency in producing carbon dioxide.*

Sand	Last five days				$\chi^2$
	+ +	+ -	- +	- -	
Peptone	2	23	10	5	15.4
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	4	14	9	3	8.1
Both media	6	37	19	8	22.9

Naturally in young cultures without protozoa where the bacterial numbers are low there is a greater production of carbon dioxide, since at this age the rapid reproduction requires a large consumption of energy which involves a rapid evolution of carbon dioxide; whereas in the presence of protozoa reproduction continues even in older cultures, resulting in a correspondingly greater production of carbon dioxide to meet the energy requirements. The rôle played by the protozoa is to reduce the number of bacteria from the beginning, and this results in a more uniform efficiency throughout the whole period; and a greater total amount of carbon dioxide is produced by smaller numbers in the same time.

From Table III it would appear that the different media are unable

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to support the same sized population: peptone supports a larger population than glucose and ammonium sulphate, yet in the latter medium the bacteria produce roughly double the amount of carbon dioxide, showing that it is not the greatest numbers that are able to produce the greatest amount of carbon dioxide.

The question arises whether, if the protozoa have a stimulating effect on carbon dioxide production by bacteria, there is any limit beyond which an opposite effect takes place. In other words, will a considerable increase in the protozoan population be followed by an increased carbon dioxide production or not? The study of such a question has experimental difficulties, but some preliminary investigations in this direction have been carried out. Experiments were made on peptone sand media with double inoculations, soil bacteria + protozoa as before followed by a second inoculation from a biological filter containing a rich culture of protozoa, especially flagellates, at different intervals, viz. (1) immediately, (2) after 2 days, *i.e.* during the maximum carbon dioxide production and (3) after 4 days. The results obtained are shown in Fig. 3 in comparison with an experiment without double inoculation. It is interesting that though in the double inoculation experiment new bacteria were introduced with the protozoa, the carbon dioxide production was decreased in every case. This decrease is in the first case less significant and agrees entirely with the result of another experiment where YB bacteria and *Colpidium* sp. were inoculated a second time. In the second and third case the repeated inoculation has not a stimulating effect, the carbon dioxide production is decreased and the final number of protozoa is always higher than that found in other experiments, as is shown in Table VI.

Table VI.

### *Final number of protozoa.*

Number of protozoa per gm. of sand

Total	Cysts	Active	Inoculation	Treatment
358,600	196,800	162,600	Protozoa and bacteria mixed culture	Sand + peptone mineral solution
421,400	236,200	185,200		
940,000	21,584	918,416	As above + protozoa and bacteria from a bio- logical filter: (a) immediately (b) after 2 days (c) after 4 days	
1,310,000	21,467	1,107,933		
1,880,000	25,061	1,674,939		
12,600	—	12,600	"YB" bacteria and <i>Colpidium</i> sp.	Sand + glucose (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> mineral solution
18,200	—	18,200		
486,400	211,200	275,200	Protozoa and bacteria mixed culture	
502,600	216,600	286,000		
18,900	—	18,900	"YB" bacteria and <i>Colpidium</i> sp.	

The results of these investigations are summarised in Table VII, from which interesting comparisons may more easily be made. The maximum amount of producible carbon dioxide calculated on the carbon

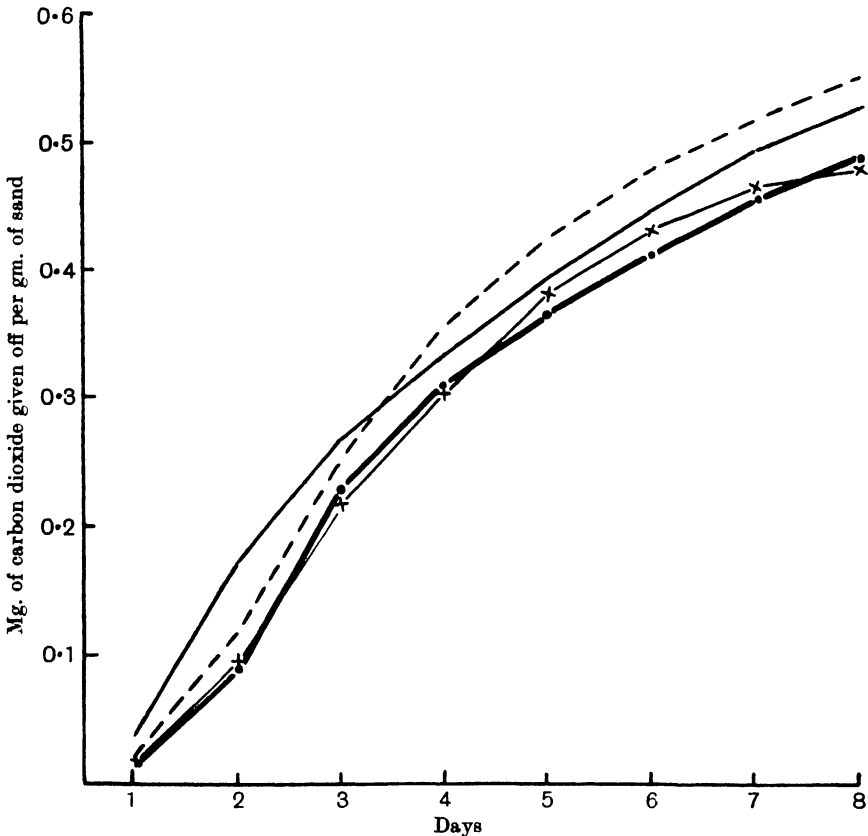


Fig. 3. Influence of repeated inoculation of protozoa. Four sand cultures used.  
 - - - - - Soil bacteria and protozoa mixed culture for all cultures.  
 — As above + second inoculation at the beginning to second culture  
 ● — ● As above + second inoculation on the second day to third culture.  
 × — × As above + second inoculation on the fourth day to fourth culture.

content of the nutrient media was taken as 100, so that the amounts of carbon dioxide actually produced could be expressed in percentages. In such a way a rough measurement is obtained for the differences in the carbon dioxide production of pure cultures and cultures with protozoa.

Table VII.

*The carbon dioxide production in 8 days and the increase in the amount of carbon dioxide by the inoculation of protozoa.*

Maximum of pro- ducible CO <sub>2</sub>	Produced amount of CO <sub>2</sub>		Inoculation	Treatment
	in mg.	in %		
440	163.2	37.09	Mixed soil bacteria culture prepared from soil	Sand + peptone mineral solution
440	169.2	38.45		
440	220.0	50.00	Protozoa and bacteria mixed culture prepared from soil	
563	285.8	50.75		
440	152.2	34.59	"YB" bacteria	
440	190.9	43.39	"YB" bacteria and <i>Colpi- dium</i> sp.	
440	201.7	45.84		
440	216.6	49.23	Protozoa and bacteria mixed culture + "YB" bacteria and <i>Colpidium</i> sp.	
			Protozoa and bacteria mixed culture and protozoa cul- ture from a biological filter:	
440	211.7	48.11	(a) immediately	
440	194.2	44.14	(b) after 2 days	
440	192.2	43.68	(c) after 4 days	
440	146.0	33.18	Mixed soil bacteria	Sand + glucose (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> mineral solution
440	253.5	57.61	Protozoa and bacteria mixed culture	
440	253.4	57.59		
440	142.6	32.41	"YB" bacteria	
440	148.7	33.80	"YB" bacteria + <i>Colpidium</i> sp.	
440	220.5	50.12		

As it appears from Table VII the glucose ammonium sulphate media gave greater differences with the same C/N ratio than the peptone media, though according to Table III the bacterial population was more numerous in the latter case. The differences between the percentage carbon dioxide production of different cultures on the same medium are as follows:

In peptone:		%
Soil bacteria and protozoa mixed culture	Mixed soil bacteria	12.60
"YB" with <i>Colpidium</i> sp.	"YB" bacteria	10.02
In glucose and ammonium sulphate:		
Soil bacteria and protozoa mixed culture	Mixed soil bacteria	24.48
"YB" with <i>Colpidium</i> sp.	"YB" bacteria	17.02

It will be seen that on glucose-ammonium sulphate media, not only the absolute amount, but also the differences in the presence or absence of protozoa are nearly double those on the peptone media. Daily microscopic observation showed that the added protozoa established themselves in the media and therefore their respiration must have contributed

to the carbon dioxide production, but cannot account for the whole of the increase. But the fact that by increasing still further the number of protozoa the production of carbon dioxide is now diminished, is evidence of some other factor operating to increase the carbon dioxide production. We have seen that this factor is *not* an increase in bacterial numbers. Unfortunately no data are available for the bacterial numbers after the second inoculation owing to an accident to the mechanism of the incubator, but it is almost certain that they would have been still further decreased. It is evident that a certain biological equilibrium exists between the bacteria and protozoa the optimum conditions of which are attained with soil bacteria + soil protozoa. If this equilibrium is affected in any direction, as occurred in these experiments with the extreme cases of the addition of more protozoa and of their absence, the percentage production of carbon dioxide is reduced.

## PART II.

The previous investigations had shown that nearly double the amount of carbon dioxide was produced from glucose-ammonium sulphate media compared with that from peptone media of similar carbon nitrogen content; and also that there is a correspondingly greater difference in favour of the inoculation with bacteria + protozoa over the inoculation with bacteria alone.

No data, however, were obtained on the question of the effect of concentration of carbohydrate and of changes in the C/N ratio in the respiration of the micro-organisms. Cutler and Crump<sup>(3)</sup> made studies with 0.2 per cent. glucose with a C/N ratio of 10/1 and found an increase in respiration in the presence of protozoa, but previous workers appear to have neglected this question.

To the present writer it appeared that the importance of these two factors in soil processes was such as to call for further investigations on the influence of concentration of carbohydrate and of different C/N ratios on respiration in the presence or absence of protozoa.

## METHODS.

The experiments were carried out in a similar manner to the previous ones, viz. sterile sand containing the nutrient media was inoculated with various cultures and the carbon dioxide produced was measured by Pettenkoffer's method. Bacterial counts were made daily and the presence of protozoa was controlled microscopically as described in detail in the previous section.

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On account of the fixed C/N ratio of peptone it could only be used for investigations on the effect of changes in concentration. It was therefore necessary to confine these studies to the use of glucose and ammonium sulphate. Mineral salt solutions containing 0.2 and 0.6 per cent. glucose were prepared, and separately sterilised ammonium sulphate solutions were added so as to give C/N ratios of 20/1, 10/1, 5/1 and 3.5/1. For the inoculations the following cultures were used, viz. (a) bacteria + protozoa from Barnfield farmyard manured plot, (b) mixed soil bacteria prepared as previously mentioned, (c) pure cultures of "YB" bacteria.

### RESULTS.

In the first experiment solutions of 0.2 per cent. glucose with ammonium sulphate equivalent to C/N ratio of 20/1, 10/1, 5/1 and 3.5/1 were inoculated with mixed cultures of protozoa and bacteria. The production of carbon dioxide calculated in mg. per gm. of sand and numbers of bacteria are plotted as curves in Fig. 4.

The curves for carbon dioxide production reached a maximum on the second day, followed by the curves of bacterial numbers a day later, in all cases except the C/N ratio 20/1, in which the two curves revealed a maximum simultaneously. With an increase of the C/N ratio the production of carbon dioxide increased.

The second experiment used the same media but omitting the C/N ratio 5/1. The inoculation consisted of mixed soil bacteria without protozoa. The results are plotted in Fig. 5.

Comparing these curves with the previous curves in Fig. 4 not only is the total amount of carbon dioxide less but the difference between the curves of the various C/N ratios is not significant.

With regard to the curves of bacterial numbers the normal curve is shown only by the one with C/N ratio of 3.5. In the case of the curves of decreasing C/N ratio there are two maxima.

In the third experiment 0.6 per cent. glucose was used with C/N ratios of 20/1, 10/1 and 3.5/1 inoculated with soil bacteria without protozoa.

The results are shown in Fig. 6. These curves of carbon dioxide production are remarkable in showing no significant differences between total yields from the various C/N ratios. The curves for bacterial numbers show two maxima in the case of C/N ratio 20/1, with the C/N ratio 10/1 the bacterial numbers and carbon dioxide production reach their maxima simultaneously and in the case of the C/N ratio 3.5/1 the bacterial numbers reach a maximum a day later.

This experiment was repeated under similar conditions using an inoculation of "YB" bacterium in pure culture.

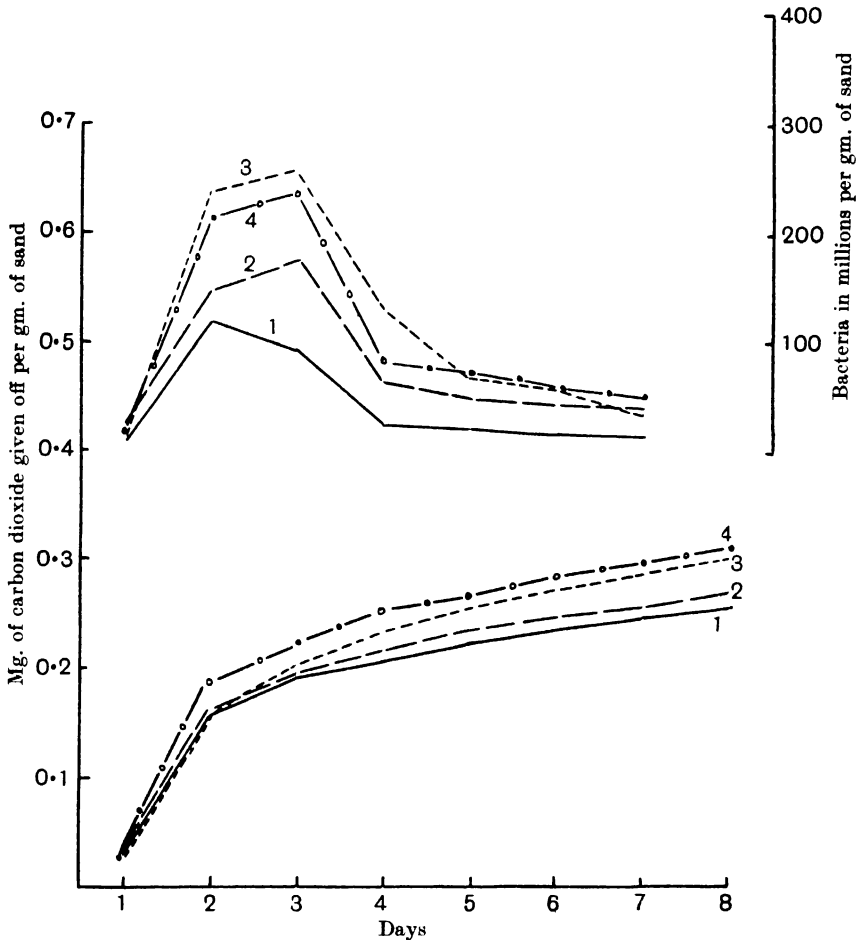


Fig. 4. Influence of C/N ratio on the  $\text{CO}_2$  production. 0.2 per cent. glucose, soil bacteria and protozoa mixed culture.

— (1) C/N=20/1.                      - - - - (2) C/N=10/1.  
 - - - - (3) C/N=5/1.                      ○—○—○ (4) C/N=3.5/1.

The results shown in Fig. 7 are generally similar to the previous results except that the increasing of the C/N ratio reduced slightly the carbon dioxide production. The culture itself also appeared to have been less efficient than the mixed population used in the previous experiments.



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The bacterial curves for the greater C/N ratio give the normal curve, but the lesser ratios give greater fluctuations.

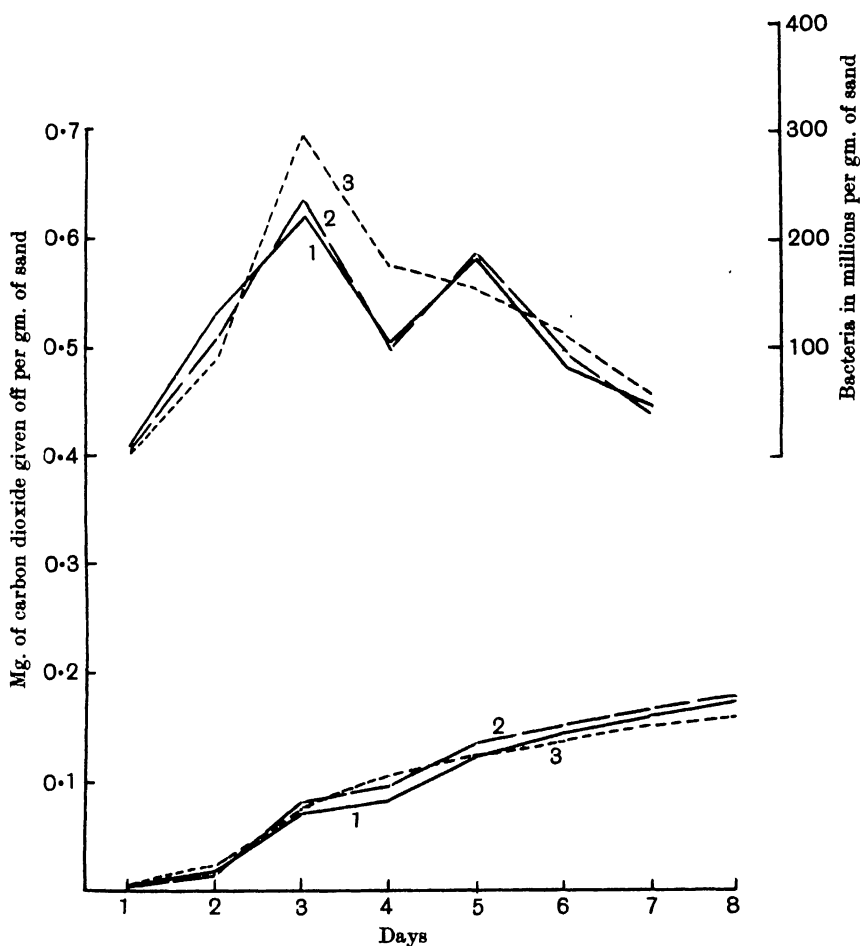


Fig. 5. Influence of C/N ratio on the CO<sub>2</sub> production. 0.2 per cent. glucose, soil bacteria alone.

—— (1) C/N=20/1.    - - - (2) C/N=10/1.    - - - - (3) C/N=3.5/1.

The fifth experiment was carried out with mixed bacteria and protozoa and the same media as in experiment three, viz. 0.6 per cent. glucose and C/N ratios 20/1, 10/1 and 3.5/1. Increasing the C/N ratio resulted in increased carbon dioxide production, and the total amount of carbon dioxide produced compared with that obtained in the previous experiments with bacterial cultures alone is much greater (Fig. 8).

The bacterial curves show less fluctuations and reach a maximum later in the narrower C/N ratios of 10/1 and 3.5/1.

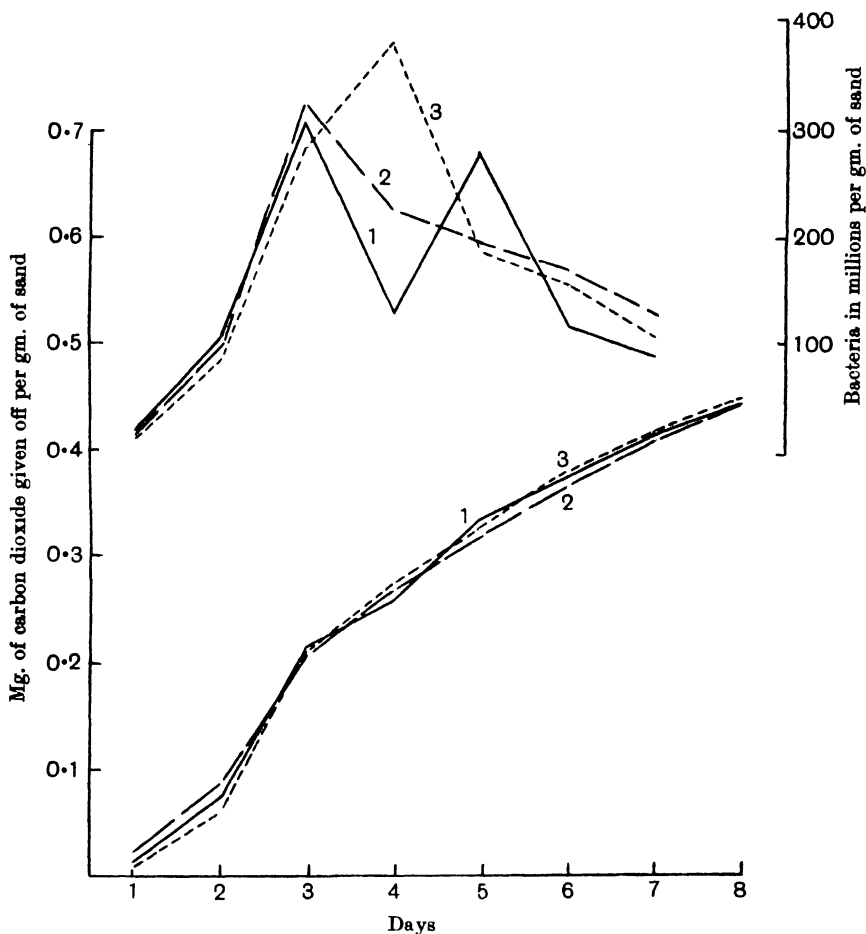


Fig. 6. Influence of C/N ratio on the CO<sub>2</sub> production. 0.6 per cent. glucose, soil bacteria alone.

—— (1) C/N=20/1.    - - - (2) C/N=10/1.    - - - - (3) C/N=3.5/1.

The results of all these experiments are collected in Table VIII, in which the maximum amount of carbon dioxide producible from the sugar is expressed as 100 and the amounts of carbon dioxide actually obtained are given as percentages.

Table VIII.

*The carbon dioxide production in 8 days and the increase in the amount of carbon dioxide by the inoculation of protozoa.*

Maximum of producible CO <sub>2</sub>	Produced amount of CO <sub>2</sub>		Inoculation	Treatment	
	in mg.	in %			
146.5	68.9	47.03	Mixed soil bacteria culture prepared from soil	Sand—mineral solution 0.2 % glucose and different amounts of (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	C : N 20 : 1
	69.8	47.65			C : N 10 : 1
	63.4	43.28			C : N 3.5 : 1
	101.5	69.28	Protozoa and bacteria mixed culture prepared from soil		C : N 20 : 1
	106.9	72.82			C : N 10 : 1
	120.3	82.11			C : N 5 : 1
	124.3	84.85			C : N 3.5 : 1
440	177.9	40.43	Mixed soil bacteria	Sand—mineral solution, 0.6 % glucose and different amounts of (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	C : N 20 : 1
	177.6	40.41			C : N 10 : 1
	170.5	40.80			C : N 3.5 : 1
	172.1	39.11	"YB" bacteria		C : N 20 : 1
	167.9	38.16			C : N 10 : 1
	165.4	37.59			C : N 3.5 : 1
	277.0	62.95	Protozoa and bacteria mixed culture		C : N 20 : 1
	289.6	65.82			C : N 10 : 1
	296.5	67.38			C : N 3.5 : 1

Comparing the two sections of the table for total carbon dioxide production it will be seen that:

(1) In every case the total percentage of carbon dioxide from similar cultures is greater with 0.2 per cent. glucose solution than with 0.6 per cent.

(2) With 0.2 per cent. glucose varying the C/N ratio from 20/1 to 3.5/1 results in an increase in total carbon dioxide production in favour of the presence of protozoa of from 22.25 to 45.57 per cent.

(3) But, with 0.6 per cent. glucose similar variation in the C/N ratio showed an increase in total carbon dioxide production in the presence of protozoa of 22.52–26.58 per cent., showing that at a higher concentration of carbohydrate the stimulating effect of the protozoa on respiration is less marked.

Finally, comparing the figures for carbon dioxide production of bacterial cultures alone, it was found that varying the C/N ratio from 20/1 to 3.5/1 did not result in any significant difference in the carbon dioxide production, the tendency, if any, was in the direction for slight reduction especially in the case of the pure cultures of "YB" bacteria.

In the first part of the paper it was shown that comparing cultures in 0.5 per cent. ~~peptone~~ and 0.6 per cent. glucose-ammonium sulphate of equivalent C/N ratio (3.5) and carbon content, the presence of protozoa caused a greater increase in the amount of carbon dioxide produced from the glucose than from the peptone media.

The second part of these investigations showed that this effect is still more pronounced with lower concentrations of glucose at all investigated C/N ratios.

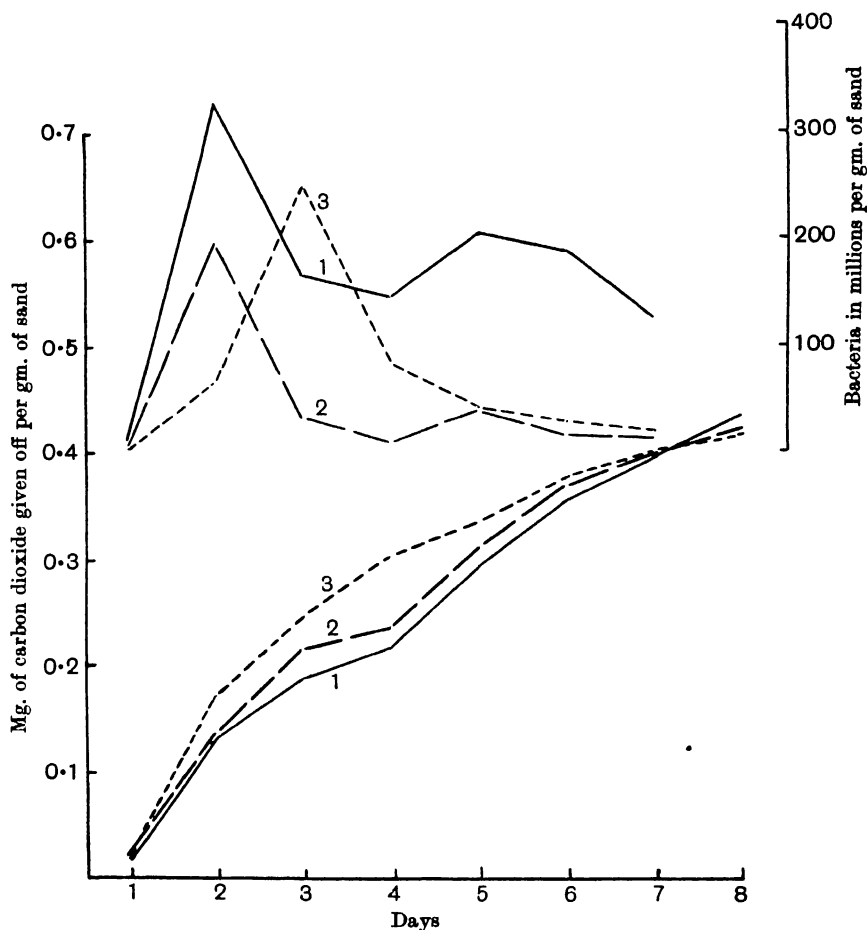


Fig. 7. Influence of C/N ratio on the CO<sub>2</sub> production. 0.6 per cent. glucose, "YB" bacteria pure culture.

— (1) C/N = 20/1.    - - - (2) C/N = 10/1.    - - - - (3) C/N = 3.5/1.

It has been shown that in bacterial cultures without protozoa the increase of the C/N ratio had no effect or only a slight depressing effect on the carbon dioxide production, whereas in the presence of protozoa there is a very pronounced increase.

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From a consideration of the bacterial curves it is seen that the correlation which normally exists between carbon dioxide production and bacterial numbers in pure cultures is of a less degree in the presence

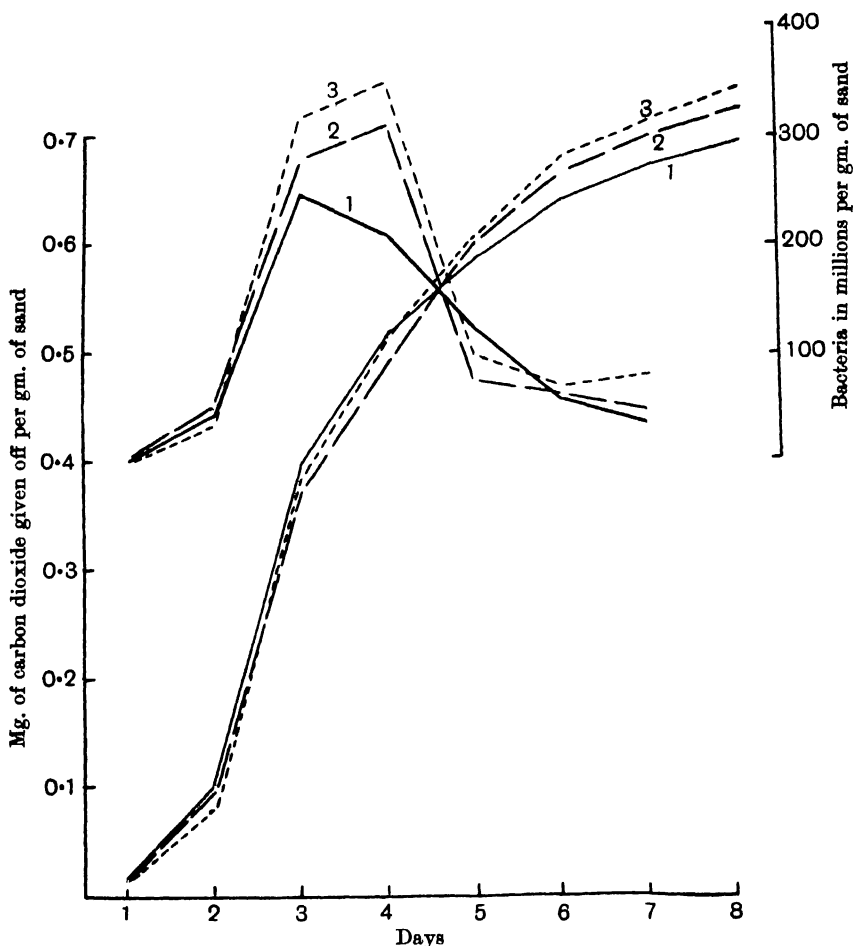


Fig. 8. Influence of C/N ratio on the CO<sub>2</sub> production. 0.6 per cent. glucose, soil bacteria and protozoa mixed culture.

— (1) C/N=20/1.    - - - (2) C/N=10/1.    - - - - (3) C/N=3.5/1.

of protozoa in glucose-ammonium sulphate media. In the latter case the density of the bacterial population is almost the same as without protozoa, whilst the carbon dioxide production is actually much greater.

In bacterial cultures either of pure "YB" or mixed soil bacteria, the correlation between numbers and carbon dioxide production is disturbed

with a C/N ratio less than 10/1. As already pointed out with a low C/N ratio the rise in bacterial numbers shows two maxima.

These fluctuations are accounted for by the shortage of nitrogen which temporarily arrests the bacterial growth until a re-assimilation of the combined nitrogen from dead protoplasm can take place resulting in a second rise in bacterial numbers with a corresponding carbon dioxide production.

In this connection it is interesting to note that on percolating filters with solutions of 0.2 per cent. sucrose-ammonium sulphate Barritt<sup>1</sup> observed that the nitrogen requirement of a mixed population was supplied by a C/N ratio of 15, and that with a wider C/N ratio the percentage purification was reduced.

#### SUMMARY.

Experiments have been carried out on carbon dioxide production from sand cultures with peptone and glucose solution inoculated with various types of bacteria and protozoa, and with glucose and ammonium sulphate solutions of varying concentrations and C/N ratio. The following results were obtained:

1. The presence of protozoa increases the carbon dioxide production especially in mixed bacterial cultures.
2. The increase of carbon dioxide production is greater in glucose solution than in peptone.
3. A further increase in the number of protozoa has an unfavourable effect on the carbon dioxide production.
4. The number of bacteria is smaller in the presence of protozoa than in their absence, but the bacterial efficiency is greater and more uniform.
5. The bacterial numbers and carbon dioxide production are definitely correlated in peptone, but in glucose to a less degree especially in the presence of protozoa.
6. The reduction of concentrations of glucose from 0.6 to 0.2 per cent. resulted in a greater percentage production of carbon dioxide.
7. With a lower concentration (0.2 per cent.) of glucose the presence of protozoa causes a greater increase in carbon dioxide production than in higher concentrations (0.6 per cent.).
8. In the absence of protozoa increasing the C/N ratio had no or only a slight depressing effect on carbon dioxide production.
9. In the presence of protozoa increasing the C/N ratio is followed by a marked increase in carbon dioxide production.

<sup>1</sup> *Biochem J.* 1931, xxv, 4, 187, 1419.

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10. In bacterial cultures a lessening of the C/N ratio below 10/1 results in a fluctuation of bacterial numbers.

I am indebted to Sir John Russell for his kindness in giving me facilities to carry out the work in this Institution. The work was done in Mr D. Ward Cutler's department, and I take this opportunity of expressing my gratitude for his ever ready help and unfailing kindness. For the culture of *Colpidium* I am greatly indebted to Miss Jane Meiklejohn of the same department.

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(Received April 23rd, 1931.)

# ON *ATOMARIA LINEARIS* STEPHENS (COLEOPTERA, CRYPTOPHAGIDAE) AND ITS LARVAL STAGES

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(With 4 Text-figures.)

## I. INTRODUCTION.

*ATOMARIA LINEARIS* Stephens has been recognised for many years as an occasionally serious pest of mangolds. Curtis (1860) notices a record made by M. Bazin in 1839 and a few years later one by Macquart, who stated that fields of red beet near Lille were completely destroyed. There does not appear to exist in the literature, however, any account of its developmental stages, nor are its breeding habits definitely known. On account of the increased difficulty of finding the adult beetles around the young mangold plants as the season advances, *i.e.* toward the end of June, the assumption has been made that they migrate to other plants and perhaps breed there. The present writer has obtained the larval stages of the beetle by placing imagines upon growing mangold plants kept in the laboratory. The object of this article is to place on record a description of the larva so that it may be recognised in the field. It is hoped to deal more fully with the bionomics of *Atomaria* in a later paper.

## II. GENERAL.

Miss Ormerod, who apparently gave the name "pigmy mangold beetle" to this insect, states in her report for 1892 that for some years previously enquiries had been sent to her regarding the nature of a very injurious attack on young mangolds. She had not at that time discovered the cause, but in that year Prof. Harker, of the Royal Agricultural College, Cirencester, communicated to her that he had, the year before, identified similar damage to mangolds with attacks by *Atomaria linearis*. He, therefore, appears to have made the first economic record of this beetle for England. That it was a well-known pest at this time on the Continent is evident from various accounts, *e.g.* that of Henze (1861) and Ritzema Bos (1891). Miss Ormerod gives further accounts of the



beetle in her reports for 1895 and 1898 and suggests measures for control, two of which are thick seeding and rotation—as they are to-day. Although a notable feature of all the attacks mentioned is the enormous numbers of beetles present, she remarked in 1898 that “up to the present date, the attack has been so rarely observed in this country that it does not appear of much practical importance beyond pointing out to us to be ready to meet it if it should occur to a greater extent.” Further outbreaks were noticed by Theobald in 1904, 1906, 1908, 1910, 1911, etc., the counties involved being Kent, Devon, Buckingham, Huntingdon, Somerset and Shropshire. With the introduction of the sugar-beet industry the beetle has found a new host, and is now frequently recorded in the monthly reports of the Ministry of Agriculture. No doubt its numbers have been considerably increased by continuous cropping with mangolds or sugar-beet, and one writer, in Czechoslovakia, refers to a recent attack as being the most severe in 20 years, as a result of a sequence of sugar-beet crops.

It will be noticed that Theobald's records are all from southern or western England. This would not seem to be entirely due to the location of this observer, since Fowler (1912) gives no record north of the Manchester district. On the Continent the beetle is a fairly frequent pest of sugar-beet, Rambousek (1926) in Czechoslovakia stating that 80 per cent. of the damage ascribed to Elaterids is due to its depredations.

### III. NATURE OF ATTACK AND HABITS OF THE BEETLE.

The most serious damage to the mangold plant is caused by the adult beetle biting the “shoots” of the seeds as they germinate. Later on, the small tap root is pitted with characteristic holes which later turn black and, in bad cases, it is eaten completely through. Often, however, though completely ringed, the central tissues may be left intact, so that outwardly the plants appear healthy and not retarded. Such plants are easily broken off. Small irregular holes are often eaten in the leaves, but this damage is usually quite insignificant.

As the plant increases in size, the root attack becomes less dangerous, provided that good growing weather prevails. Beetles may be found at the same time both in the crown of the plant and in the surface layer of soil. When disturbed they feign death, and may be easily overlooked, unless given time to resume their activities. As was mentioned by Dowling (1908) the beetles were not found to be more active at night. This writer also refers to the apparent disappearance of the beetles, as the mangolds grow bigger, and states that by mid-June hardly a beetle

could be found. He often noted them about this time or a little later, flying in large numbers in clearings in a wood some  $\frac{1}{2}$  mile from the nearest mangolds, and suggested that a migration had taken place.

In 1930 this apparent early disappearance was observed by the present writer also, but during 1931 an observation was made that may account for it in another way. In a plot of sugar-beet, a few beetles per plant could be collected early in the season by examining a number of consecutive plants. Later, at the end of June, a similar procedure produced no beetles at all—unless one was fortunate enough to find an individual plant which, for some unknown reason, proved to be specially attractive. In such cases the surrounding soil suggested the activities of a small ants' nest. It was swarming with beetles and numbers up to a hundred were counted. It is thought that the beetles may congregate at this period in numbers about individual plants—perhaps for breeding purposes—leaving the other plants free. Theobald reported that the numbers of beetles decreased about the end of July, in one year as late as the middle of August.

With regard to the amount of damage the beetles are capable of doing, it was found in the laboratory that a single one will prevent the development of a seedling that has just germinated, whereas, when the full cotyledon stage has been attained, the depredations of many did not noticeably affect it.

In addition to the cultivated mangold and sugar-beet, the observations of Lügenbuch and Schewket (1931) indicate that *Atomaria linearis* has other food plants in spinach, radish, marjoram, *Chenopodium album*, *Stellaria media* and *Polygonum aviculare*.

#### IV. REVIEW OF CONTROL MEASURES.

Theobald claimed good results from cross ring-rolling and so consolidating the soil around the seedlings. In this connection an observation of Dowling is of interest. He noted that, in a field severely attacked by *Atomaria*, a small area stood out as noticeably healthy. On inquiry it was found that a mistake had been made—rolling had been begun while the field was still too wet. Some amount of panning had therefore occurred and the beetles could not get to the plants. A similar effect of panning due to flooding was observed on an area of Barnfield on the Rothamsted Farm in 1931, but, in this case, the panning was so complete that the plants themselves were badly retarded. As the worst aspect of the damage is the early attack in the germinating stage, sowing deterrents with the seed is an obvious measure, and many Continental

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writers claim good results from this procedure. For example, the use of 1 lb. of naphthalene to 20 lb. of seed has been advised. In this country, in a recent report of research work (1931), the use of carbolic acid 1 per cent. and magnesium sulphate 5 per cent. is advocated as a seed steep, but the advantage of using the latter ingredient is by no means evident.

Lägenbuch and Schewket (1931), however, did not find that naphthalene treatment was of any value and state that spraying with nicotine is the only measure of use. Another spray fluid said to be effective, in Czechoslovakia, is a 1 per cent. solution of sodium or potassium cyanide. Rambousek (1926) suggests the trapping of the beetles in early autumn by means of holes in the ground suitably baited with sugar beet. In Denmark thick sowing and late thinning out is advised as a suitable measure and, from the observations made by the present writer during 1930-31, this would seem quite effective, though the attacks observed were not heavy.

### V. GENERAL REMARKS ON THE LIFE HISTORY.

It seems to be established that *A. linearis* hibernates as an adult in soil (Morris, 1927) or among debris. Dowling records it as attacking April-sown mangolds. It first came under observation by the writer in late May attacking mangolds, and in early June on sugar beet. Copulation was to be seen occurring in early June, and couples were frequently taken throughout the month and during the first week of July. It was observed to last for over 2 hours in some cases and was seen to take place only in the soil. Theobald (*loc. cit.*), however, notes pairing as occurring on the wing. As already stated the beetles were difficult to find after June, and in the laboratory they began to die off at the beginning of August. Rambousek refers to finding enormous numbers in early autumn in debris of beet fields, and presumably this is the new generation. There would, therefore, appear to be only one brood in the year.

#### *Laboratory observations.*

It is difficult to induce *Atomaria* to oviposit in captivity, a fact already noted by Theobald and by Dowling who both failed to obtain eggs. A number of devices were tried in order to facilitate the search for the necessarily minute eggs and young larvae. Moist black filter paper was used instead of soil; plants were grown in tubes of water-culture solution, allowing the beetles access to the upper regions of the plant only, by passing it through a cotton-wool-plugged hole in a cork, the lower part of the root system being immersed in the solution; other plants were

placed in tubes embedded in a block of plaster of Paris that was kept moist in a bath of water-culture solution; still others in open-ended tubes, plugged with cotton-wool and containing a minimum quantity of soil, the whole being sunk into a pot of soil. A few eggs were obtained by these means and one or two were recovered by washing small quantities of infected soil. It was found, however, that normally grown plants were best for later stages, and the larvae were recovered from them by suspending the soil on wire gauze over water. Both mangold and sugar beet were used as host plants, but actually the larvae were recovered from mangold plants.

The eggs took from 4 to 6 days to hatch under laboratory conditions. From a pot set up out of doors in the middle of June, half-grown larvae were recovered during mid-July and fully grown larvae early in August. The egg and larval stages occupy, therefore, at a maximum, a period of 6 weeks.

The larvae apparently live freely in the root system of the plants. Occasionally the Malpighian tubules are red in colour, resulting, it is believed, from the ingestion of red pigment of the epidermal layers of the upper part of the tap root of sugar beet.

*Description of the egg and larval stages.*

Few Cryptophagid larvae have been hitherto described and only one of these belongs to the genus *Atomaria*. The species concerned is

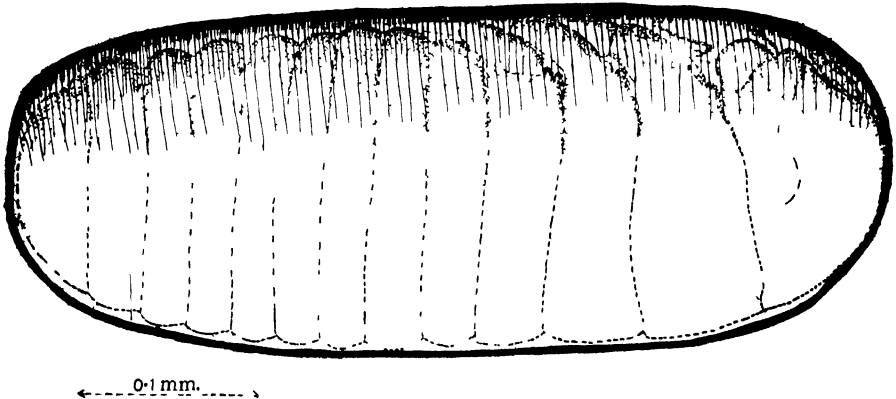
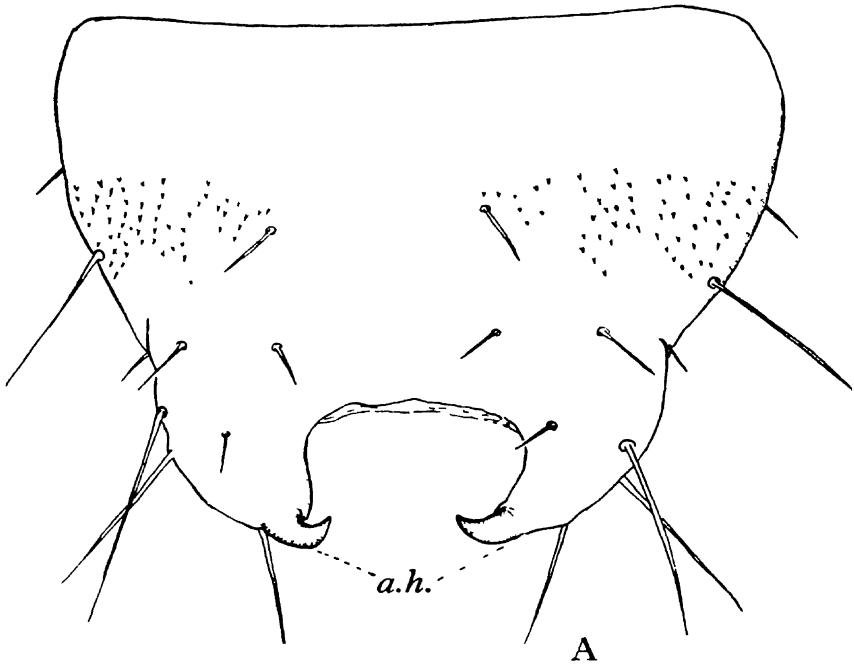


Fig. 1. The egg with developing larva inside.

*A. nigripennis* Payk., of which an inadequate account is given by Erichson (1848). The larva of *A. linearis* is, therefore, described in some detail.

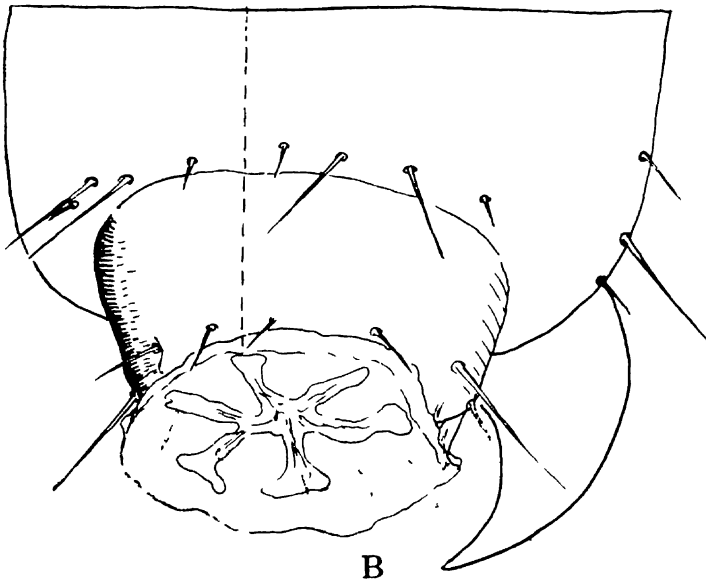
The egg (Fig. 1) is elongate-oval, measuring just under  $\frac{1}{2}$  mm. long



A

Fig. 2. A, last abdominal segment of fully grown larva from above.  
a.h., anal horns.  $\times 300$ .

*mid.v.*



B

Fig. 2. B, from beneath, slightly from one side.

and about 0.18 mm. in width. The colour is translucent greyish and there is no sculpturing.

The newly hatched larva is about  $\frac{1}{2}$  mm. long and 0.15 mm. across. It differs from later stages in that, relative to body size, the head, spiracles, claws, and anal horns are very much larger. The setae also are

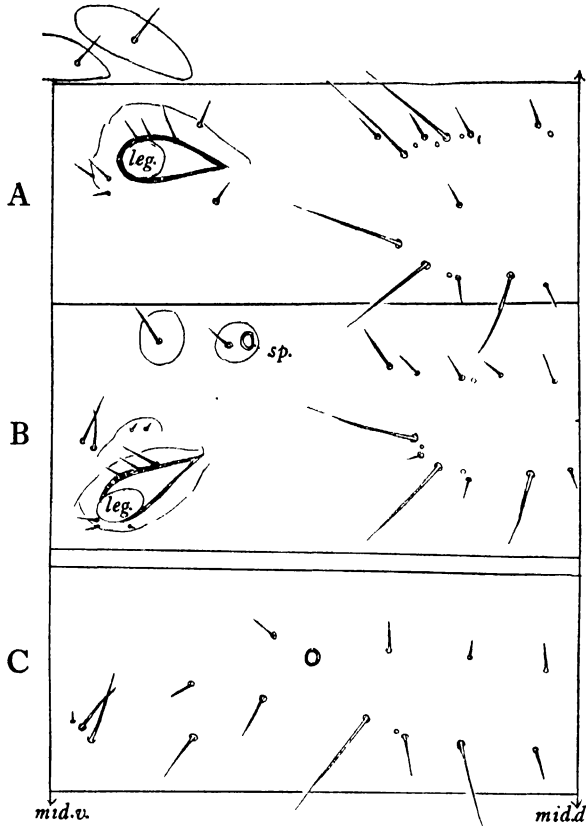


Fig. 3. Map of setal arrangement on A, prothorax; B, mesothorax; C, abdominal segments 1-8. *leg.*, section of insertion of leg; *mid.v.*, mid-ventral; *mid.d.*, mid-dorsal line. *sp.*, spiracle.

proportionately much larger and give the larvae a spiny appearance which is lost later on. The arrangement of the setae is, however, essentially similar in all stages and is described for the fully grown larva.

The fully grown larva (Fig. 4 A) is just under 3 mm. long, some 0.4 mm. across, and of a translucent greyish colour. Sclerotisation is weak, the head capsule and anal horns being scarcely coloured. The three thoracic segments bear three pairs of weakly chitinised legs (Fig. 4 G),

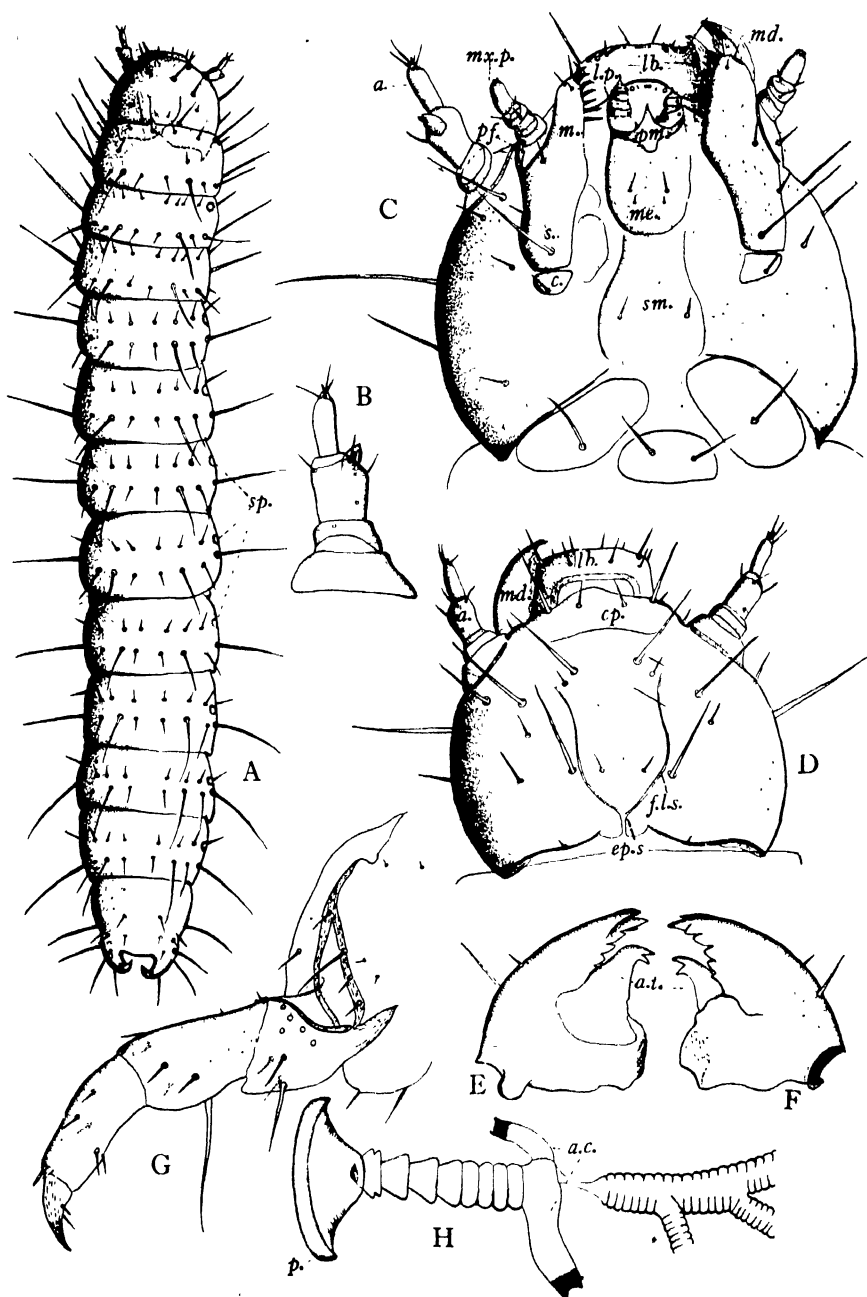


Fig. 4.

well armed with spines and ending in a strong terminal claw. There are nine abdominal segments of which the last is narrowest. This carries dorsally two inwardly and upwardly directed, curved, seta-bearing horns and ventrally, a papilla carrying the anus. This is presumably the reduced tenth segment judging from its setae. The mesothorax and first eight abdominal segments each bear a pair of spiracles.

The arrangement of the setae on the prothorax differs from that on the last two thoracic segments. On the abdomen the setal arrangement is alike on the first eight segments but that on the ninth segment (Fig. 2) exhibits certain differences.

A chart showing the disposition of the setae on the prothorax, mesothorax and abdominal segments 1-8 is given in Fig. 3.

It will be noticed that between head and prothorax in the ventral position there is a median unpaired plate and two lateral plates which may correspond to the spiracular and its accompanying plate shown in the other thoracic segments. Each of the body segments shows a median dorsal suture.

The head (Fig. 4 C and D) is approximately circular in outline and somewhat flattened, with the epicranial and fronto-lateral sutures well-developed posteriorly, while indications of ante-clypeal and post-clypeal sutures are present in front. The setal arrangement is shown in the figures. The antenna (Fig. 4 B and *a.*, Fig. 4 C and D), has three joints excluding the base; the second segment is the largest and carries ventrally and externally a small conical lobe as well as the elongate last segment. The antenna thus has a biramous appearance. The last two segments carry three spines and two peg-like structures.

The labrum (*lb.*, Fig. 4 D) bears two transverse rows of setae and the anterior margin is armed with short spines. The mandibles (Fig. 4 E

Fig. 4.

- A. Dorsal view of larva, about half-grown.  $\times 60$ .
  - B. Dorsal view of right antenna.  $\times 250$ .
  - C. Ventral view of head capsule of fully grown larva, with right mandible removed.  $\times 180$ .
  - D. Dorsal view of same.  $\times 140$ .
  - E. The right mandible ventral aspect.  $\times 260$ .
  - F. The same, dorsal aspect.
  - G. Right metathoracic leg, inner aspect.  $\times 250$ .
  - H. Spiracle from abdominal segment in side view.  $\times 900$ .
- a.* antenna, *a.c.* arms of closing apparatus, *a.t.* auxiliary tooth, *c.* cardo, *cp.* clypeus, *ep.s.* epicranial suture, *f.l.s.* fronto-lateral suture, *lb.* labrum, *l.p.* labial palp, *m.* mala, *md.* mandible, *me.* mentum, *mx.p.* maxillary palp, *p.* peritreme, *pf.* palpifer, *pm.* prementum, *s.* stipes, *sm.* submentum, *sp.* spiracles.



and F) have two main teeth, the dorsal inner surface of the upper tooth having serrations or smaller teeth. In addition there is an auxiliary tooth (*a.t.*) arising from the inner side of the molar area and ending in two strong hooked denticles.

The maxilla consists of a single lobe, the mala (*m.*), and a reduced maxillary palp (*mx.p.*). The palp consists of three segments and a basal area, the palpifer (*pf.*). The penultimate segment carries two small spines and the apex of the last segment is beset with a group of papillae; on the dorsal aspect of the last segment is a long peg-like structure. Cardo (*c.*) and stipes (*s.*) are present, the first-mentioned sclerite being small.

The labium is considerably reduced, consisting of two-jointed peg-like palps (*l.p.*), a prementum (*pm.*) and only indications of mentum and submentum (*m.* and *sm.*).

The tracheal system presents no features of special interest. There are present the usual mesothoracic and eight abdominal pairs of spiracles. All are of similar structure, the mesothoracic spiracle differing only in its slightly larger size and its more ventral position. The peritreme (*p.*, Fig. 4 H) is circular and surrounds a cup-shaped depression, at the bottom of which is a slight protuberance. This carries the actual opening of the spiracle and widens out into a short tube provided at its base with two projecting arms, one longer and one shorter (*a.c.*, Fig. 4 H). These are part of the closing mechanism and serve for the attachment of the muscles concerned.

Below the arms is a constriction which opens out into the main tracheal tube.

## 6. SUMMARY.

1. A brief survey is made of the history and habits of *Atomaria linearis* Stephens, the Pigmy Mangold Beetle, and some new observations recorded.

2. The egg and the external structure of the larva are described for the first time.

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(Received September 14th, 1931.)

# STUDIES UPON THE COPPER FUNGICIDES

## I. THE INTERACTION OF COPPER SULPHATE WITH CALCIUM HYDROXIDE

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(With 4 Text-figures.)

NUMEROUS sprays in which the toxic action of copper derivatives upon fungi is utilised for the control of plant diseases caused by copper-sensitive fungi have been proposed but few have met with success. For most fungi against which these materials are employed, mycological data are available to place factors such as correct time and frequency of application upon a scientific basis. It is difficult therefore to attribute failure to incorrect use, and it is probable that success is due to chemical and physical factors, to examine which is the main purpose of the work to be described in this series. For knowledge of the chemical nature of the successful copper fungicides, of the changes the copper-containing deposit upon the plant undergoes after spraying and of the mechanism whereby fungus attack is prevented is scarce and to those questions which have been examined, various hypotheses supply conflicting and unsatisfactory answers.

Attention was given in the first place to Bordeaux mixture, for this, the oldest of the copper-containing sprays, has survived in spite of the inconveniences of its preparation. Bordeaux substitutes in paste or powder form have often been tried, but all appear to lack some of the chemical and physical properties which go to make the efficiency of Bordeaux mixture. To determine these properties it is necessary to ascertain the chemical character of the precipitate of Bordeaux mixture—the product of the interaction of copper sulphate and calcium hydroxide solutions.

### THE PRECIPITATION OF CUPRIC SULPHATE SOLUTIONS BY CALCIUM HYDROXIDE.

The generally accepted views upon the composition of the precipitate of Bordeaux mixture are derived almost entirely from the classical research of Pickering<sup>(18)</sup>. He showed that the composition of the precipitate was dependent upon the amount of calcium hydroxide added per

unit of copper sulphate, and concluded that a series of basic copper sulphates and basic copper-calcium sulphates were produced as the proportions of lime were increased. In the mixture prepared from equal weights of bluestone and quicklime, the precipitate had the composition  $10\text{CuO} \cdot \text{SO}_3 \cdot 4\text{CaO} \cdot \text{SO}_3$ . When smaller proportions of lime were used, he concluded that the precipitate consisted of less basic sulphates in which the ratios  $\text{CuO} : \text{SO}_3$  were 10 : 1, 5 : 1 and 4 : 1. Pickering's work has been repeated by two later investigators, Sicard (22) and Wöber (26), both of whom have differed from him on important points. There is a general agreement, however, that the least basic sulphate contains the complex  $4\text{CuO} \cdot \text{SO}_3$  and is formed by the addition of calcium hydroxide in amounts up to 0.75 equivalent. The further addition of lime, according to Pickering, renders the supernatant solution alkaline to phenolphthalein, but until the molecular ratio of copper sulphate to calcium hydroxide reaches 10 : 9, this alkalinity disappears on standing. In his opinion this change in alkalinity was due to the interaction of the excess calcium hydroxide with the  $4\text{CuO} \cdot \text{SO}_3$  basic sulphate to form more basic compounds in which the ratio  $\text{CuO} \cdot \text{SO}_3$  was equal to 5 : 1 and 10 : 1. Sicard, who employed similar methods, found that the point of permanent alkalinity was not reached until an equimolecular amount of calcium hydroxide had been added, at which point he found that the composition of the precipitate agreed with the formula  $5\text{CuO} \cdot \text{SO}_3 \cdot \text{CaO}$ . Wöber, on the other hand, found that permanent alkalinity is produced if lime be added in excess of the ratio  $\text{CuO} : \text{CaO} = 10 : 8$ , the precipitate then corresponding to the formula  $\text{CuSO}_4 \cdot 4\text{Cu}(\text{OH})_2 \cdot \text{H}_2\text{O}$ . In support of this conclusion he showed that the formation of calcium sulphate ceased at this point.

These investigators employed stoichiometrical methods for the establishment of the identity of the compounds they considered to be formed. Since Pickering's time the conception of a chemical compound has been profoundly modified by the application of the phase rule. Further, the introduction of electrometric methods of determining hydrogen-ion concentration has not only provided a better method for the investigation of the reaction of the solution but has been used to throw light upon the phenomena of basic salt formation. For the particular problem of the chemistry of the Bordeaux precipitate belongs to the general problem of basic salt formation. It is now generally realised that the addition of alkali to solutions of metallic salts rarely results in the simple precipitation of the hydroxide. In most cases the precipitate contains a certain amount of the acidic radicle, carried down either by

adsorption or in the form of definite basic salts. The action of alkalies upon solutions of cupric salts has been employed frequently since Pickering's work for the study of this phenomenon, but, as this work is concerned mainly with the action of sodium hydroxide, it will not be discussed here except when, because of the analogy between this reaction and that of calcium hydroxide upon cupric salts, it throws light upon the chemistry of Bordeaux mixture.

For the study of the action of calcium hydroxide upon dilute copper sulphate solutions it was, however, found convenient to employ methods similar to those of Pickering, Sicard and Wöber, to interpret the results obtained after the manner of these workers and to criticise these interpretations from the view-point of modern physico-chemical theory. The general procedure has been to investigate the changes which occur when increasing amounts of lime water are added to solutions of copper sulphate with special reference to the amount of sulphate radicle remaining in solution and to the hydrogen-ion concentration of the supernatant solution.

(1) *The amount of sulphate radicle present in solution.*

Details of the experimental work are given on p. 112 and from the data there recorded it is possible to draw a graph showing the percentage of sulphate radicle remaining in solution as the proportion of calcium oxide added is increased. Fig. 1 is derived from data obtained in Exp. A, Series 2. It will be seen that the first section of the curve shows a rapid decrease in the amount of sulphate radicle in solution to a point at which the amount of lime added corresponds to the ratio  $\text{CuO} : \text{CaO} = 1 : 0.75$ , at which point the whole of the copper initially present has been precipitated. If it be assumed that the precipitate consists of the  $4\text{CuO} \cdot \text{SO}_3$  basic salt the theoretical amount of sulphate radicle converted to calcium sulphate is indicated by the dotted line in the graph. That in many cases the individual determinations give points falling slightly below this line indicates a slight adsorption of the copper and calcium sulphates in solution by the precipitate. The analytical figures, given in Table II, for the amounts of copper and sulphate sulphur in the precipitate indicate that it is the  $4\text{CuO} \cdot \text{SO}_3$  basic salt.

The conclusion, that when an amount of calcium hydroxide less or equal to that which gives complete precipitation of the copper is added, the precipitate consists of the  $4\text{CuO} \cdot \text{SO}_3$  basic sulphate, is in agreement not only with Pickering, Sicard and Wöber, but also with the findings of those investigators who employed alkali hydroxide solutions instead of calcium hydroxide, *e.g.* Proust (21), Smith (23), Field (11) and Williamson (25).

More recently, however, Nelson (17) obtained indications that the initial compound precipitated by the addition, at ordinary temperatures, of sodium hydroxide to cupric sulphate solutions had a  $\text{CuO} : \text{SO}_3$  ratio of 3.5 : 1, a conclusion which might be explained if an adsorption of sulphate by the basic precipitate occurred. In view of the possibility, which Fowles (13) considered sufficient to invalidate the method, that alkali sulphate may be carried down by adsorption with the basic sulphate, electrometric methods have been applied to determine by indirect means

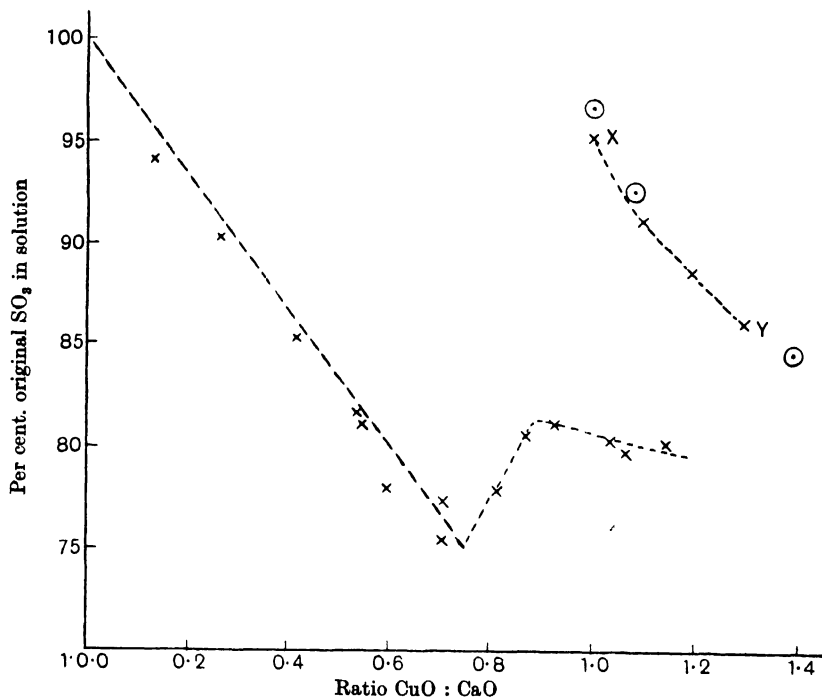


Fig. 1.

⊙ — ⊙ from data given by Bell and Cameron.

x — x data from Expt. A, Series 2.

the composition of the precipitate. If free copper sulphate be present the solution has an acid reaction due to hydrogen ions produced by the hydrolysis of the copper sulphate. At the point of complete precipitation of the copper there will therefore be a point of inflection upon the hydrogen-ion concentration curve, the position of which will not be affected by adsorption of alkali sulphate. The error introduced into the direct estimation of the  $\text{CuO} : \text{SO}_3$  ratio of the precipitate by the presence of alkali sulphate will thus be avoided. Employing an oxygen electrode,

Britton(4) found that if the sodium hydroxide be added slowly so that an amorphous precipitate is formed the hydrogen-ion concentration of the solution decreased rapidly after 1.47 equivalents of alkali had been added. He calculated that the precipitate contained amounts of copper and sulphate radicle in the molar ratio of 4 : 1. A similar conclusion is to be derived from both the quinhydrone and glass electrode studies quoted by Britton(7). Hopkins and Beebe(15) concluded that the single sharp inflection obtained with dilute solutions at room temperature indicated the formation of but one definite compound, the basic salt  $\text{CuSO}_4 \cdot 3\text{Cu}(\text{OH})_2 \cdot x\text{H}_2\text{O}$ .

The individuality of the  $4\text{CuO} \cdot \text{SO}_3$  basic sulphate has been indicated by phase rule studies of the system  $\text{CuO} - \text{SO}_3 - \text{H}_2\text{O}$ , but, owing to the time taken for equilibrium to be established, the results obtained by various workers are not in good agreement. Neither Bell and Taber(2) nor Young and Stearn(27) obtained indications of the formation of definite basic sulphates. Britton(5) concluded that, at 25° C., there is but one basic sulphate which has the composition represented by the formula  $4\text{CuO} \cdot \text{SO}_3 \cdot 4\text{H}_2\text{O}$ . Bell and Murphy(1) also showed that, at 100° C., the only basic salt formed has the composition in agreement with this formula. But in neither case does the plotting of the analytical data give a satisfactory point of intersection of the tie-lines and Bell and Murphy's conclusion is based upon the analysis of the washed precipitate. Posnjak and Tunell(20) studied the system at 50°, 100° and 200° C. and found three basic sulphates:  $3\text{CuO} \cdot \text{SO}_3 \cdot 2\text{H}_2\text{O}$ ,  $4\text{CuO} \cdot \text{SO}_3 \cdot 3\text{H}_2\text{O}$  and  $3\text{CuO} \cdot 2\text{SO}_3 \cdot 5\text{H}_2\text{O}$ . The ranges of concentration over which the  $3\text{CuO} \cdot \text{SO}_3$  and  $3\text{CuO} \cdot 2\text{SO}_3$  basic salts appear increase with temperature. On the other hand, the  $4\text{CuO} \cdot \text{SO}_3$  salt, which does not appear in the 200° C. isotherm, is more prominent at the lowest temperature. Conclusions based upon the analogy between precipitation of copper sulphate solutions by sodium hydroxide and calcium hydroxide solutions involve the assumption that the function of the alkali is to remove the acid formed by the hydrolysis of the copper sulphate. Fowles(13) obtained evidence that the final product of hydrolysis of dilute cupric sulphate solutions is the 3 : 1 basic sulphate. This salt is presumably the  $3\text{CuO} \cdot \text{SO}_3 \cdot 2\text{H}_2\text{O}$  compound whose individuality was established by Posnjak and Tunell (*loc. cit.*). There is no evidence in the sulphate curve (Fig. 1) that compounds less basic than the  $4\text{CuO} \cdot \text{SO}_3$  salt are formed and a review of the literature shows that the majority of cases in which basic sulphates less basic than the 4 : 1 salt have been obtained were by precipitation from hot solution. This conclusion is

supported by the observation of Hopkins and Beebe<sup>(15)</sup> that, when the precipitate obtained by the addition of less than 1.5 equivalents of sodium hydroxide is boiled in the presence of copper sulphate, the 4 : 1 salt is slowly converted to a less basic sulphate which they suggested may be the 3 : 1 salt. Finally, the conclusion that the 3 : 1 salt is not formed by precipitation at room temperatures is supported by Fowles'<sup>(13)</sup> observation that the 3 : 1 salt is decomposed by cold water to form the 4 : 1 salt. Pickering's conclusion, that the 4 : 1 basic sulphate is precipitated by the addition of lime water in amounts less than 0.75 equivalent, is therefore confirmed by the present work and is substantiated, not only by recent work upon the analogous sodium hydroxide precipitation, but by phase rule studies of the system  $\text{CuO} - \text{SO}_3 - \text{H}_2\text{O}$ .

Continuing the study of the fate of the sulphate sulphur when amounts of calcium hydroxide in excess of 0.75 equivalents are added, it will be seen from Fig. 1 that part of the precipitated sulphate re-enters solution. This sulphate sulphur can only be formed by the decomposition of the 4 : 1 basic salt which, according to Pickering<sup>(18)</sup> and to Sicard<sup>(22)</sup>, is converted to more basic sulphates,  $5\text{CuO} \cdot \text{SO}_3$  and  $10\text{CuO} \cdot \text{SO}_3$ . Wöber<sup>(26)</sup> also considered that the appearance of further amounts of calcium sulphate followed conversion to the 5 : 1 salt, a conclusion supported by his observation that further amounts of calcium sulphate ceased to be formed when 0.8 equivalents of calcium hydroxide had been added. From the results shown in Fig. 1 it will be seen that there is a change of direction of the curve at the point when 0.9 equivalents of alkali had been added. The sulphate sulphur content of the precipitate at this point is approximately 20 per cent., and, applying Wöber's argument, it would be concluded that the 4 : 1 basic sulphate is converted to a compound of empirical composition  $5\text{CuO} \cdot \text{CaO} \cdot \text{SO}_3$ ; a basic copper-calcium sulphate for phase rule studies have shown no evidence of the existence of a  $5\text{CuO} \cdot \text{SO}_3$  basic sulphate in the system  $\text{CuO} - \text{SO}_3 - \text{H}_2\text{O}$ .

This conclusion involves the assumption that no adsorption of calcium sulphate by this compound has occurred. The failure of the sulphate radicle recovery to approach more closely to 100 per cent. might equally well be explained by adsorption and it is possible that the direction of the latter part of the curve is controlled mainly by the solubility of calcium sulphate in the supernatant solution. That the solubility of calcium sulphate may be the controlling factor is suggested by solubility data. The experimental determination of the solubility of calcium sulphate in calcium hydroxide solutions is made difficult by the tendency to form supersaturated solutions and it was deemed inadvisable to attempt the



estimation of solubility figures except by the direct method described on p. 113. If it be assumed that, for reasons which are discussed below, free calcium hydroxide will only appear permanently in solution after the ratio  $\text{CuO} : \text{CaO} = 1 : 1$  has been passed, the curve *XY* (Fig. 1) will represent the solubility of calcium sulphate in the calcium hydroxide solutions so produced.

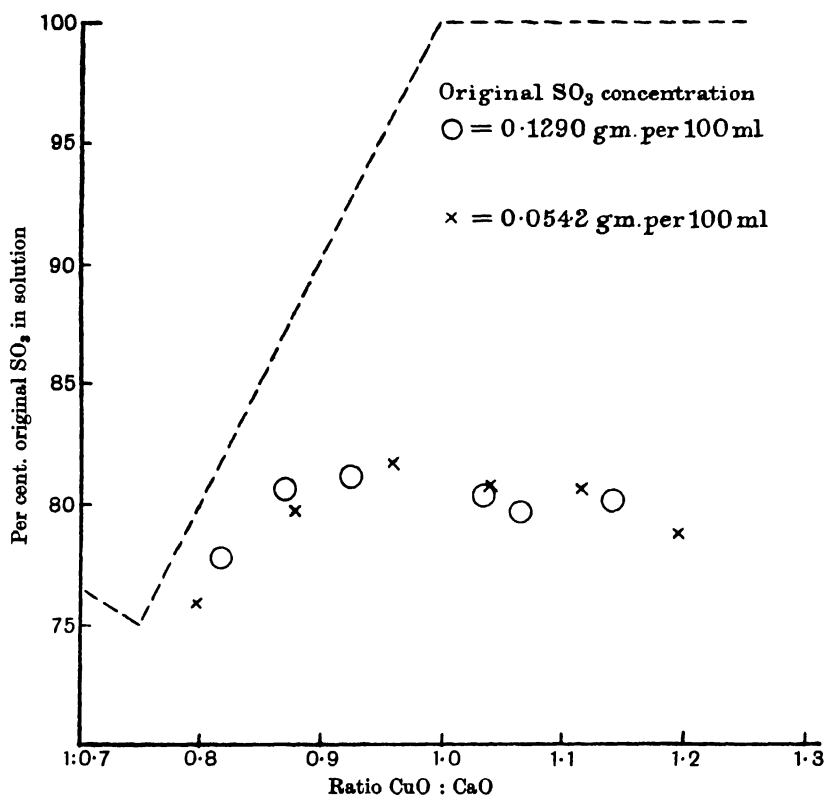


Fig. 2.

The disturbing factor introduced by the low solubility of calcium sulphate may be avoided by the use of more dilute solutions or by the examination of an analogous reaction in which a soluble calcium salt is formed. The results of a series of trials in which the initial concentration of the copper sulphate was 0.167 per cent. are illustrated in Fig. 2, the experimental data being recorded on p. 113. It will be seen that the amount of sulphate radicle in solution when amounts of lime water giving  $\text{CuO} : \text{CaO}$  ratio of between 1 : 0.75 and 1 : 1 are very similar to

those given in the first series of trials (Fig. 1), when the initial concentration of the copper sulphate was 0.25 per cent. This similarity would seem to give strong support to the view that the change of direction of the curve at the  $\text{CuO} : \text{CaO}$  ratio of 1 : 0.9 is due to the formation of a definite basic copper-calcium sulphate, but the extent of adsorption would have to be determined before any conclusion of its composition can be deduced from the analytical figures.

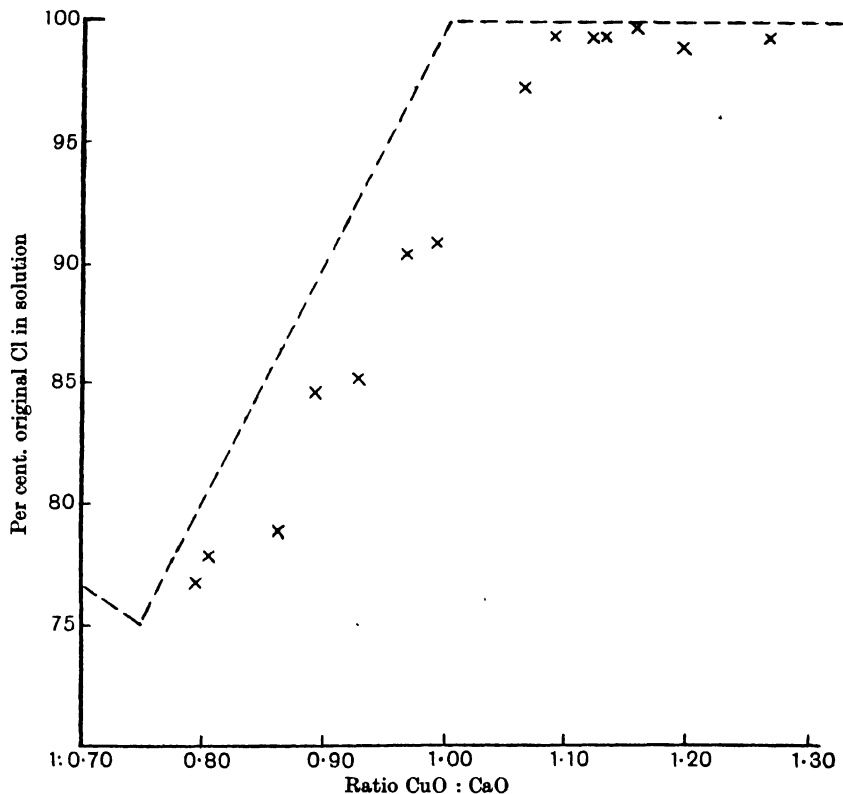


Fig. 3.

For the examination of an analogous reaction in which the calcium salt formed is of higher solubility than calcium sulphate, the interaction of copper chloride and calcium hydroxide was chosen. The results obtained are illustrated by Fig. 3, which incorporates the analytical figures given on p. 114. It is apparent that there is no break in the chloride content curve corresponding to that at 0.9 equivalents of calcium hydroxide in the sulphate curve. At 0.75 equivalents the basic chloride formed is the

4 : 1 salt and, if it be assumed that the reaction of this salt with additional calcium hydroxide involves the displacement of the chloride by the hydroxyl radicle, the theoretical amount of chloride in solution is given by the dotted line. That the actual value obtained falls below this line may be attributed to an adsorption by the precipitate of calcium chloride formed by this reaction.

This result suggests that the failure of the amount of sulphate radicle to reach nearer 100 per cent. recovery is due, not to the formation of an insoluble copper-calcium sulphate, but to adsorption of calcium sulphate by the compound produced by the interaction of lime water and the 4 : 1 basic sulphate. In dealing with the effect of adsorption of alkali sulphate upon the direct analytical estimation of the composition of the  $4\text{CuO} \cdot \text{SO}_3$  salt, it was pointed out that the indirect evidence afforded by the electrometric study of the reaction would avoid this error. In the same way an examination of the hydrogen-ion concentration of the supernatant solution as the ratio  $\text{CuO} : \text{CaO}$  approaches 1 : 1 may be used to give information of the composition of the precipitate and to avoid the error due to adsorption of alkali sulphate.

(2) *The hydrogen-ion concentration of the solution.*

Pickering<sup>(18)</sup> showed that the interaction of the  $4\text{CuO} \cdot \text{SO}_3$  salt with calcium hydroxide is indicated by changes in the hydrogen-ion concentration of the supernatant solution, as shown by the colour change of phenolphthalein. If this interaction be allowed time to attain equilibrium, the hydrogen-ion concentration curve should show inflections which serve to indicate the composition of the precipitate. If the change of direction of the sulphate sulphur curve at the ratio  $\text{CuO} : \text{CaO} = 1 : 0.9$  represents the formation of a basic copper-calcium sulphate, a point of inflection will occur on the *pH* curve at this point. If, as suggested by the study of the interaction of cupric chloride with lime water, the reaction between the 4 : 1 basic chloride and calcium hydroxide brings about a complete displacement of the chlorine from the basic chloride, the point of maximum change in *pH* will coincide with the ratio  $\text{CuO} : \text{CaO} = 1 : 1$ . Further, the formation of intermediate basic salts will be indicated by steps in the neutralisation curve which yield indirect information of the composition of such salts.

The electrometric studies of the interaction of alkalis and cupric salts by the investigators referred to in the previous section were confined to the action of sodium hydroxide. From the accounts of this work it would appear that definite alkalinity results after the addition of

1.50 equivalents of alkali. Britton (4), in his oxygen electrode titrations, showed that the  $pH$  of the solution rose rapidly to values approximating to 10 or 11 at this point, and he considered that Pickering's observation that permanent alkalinity was not reached until 1.8 equivalents had been added was due to the manner in which the alkali had been added. Hopkins and Beebe (15), who used the quinhydrone electrode, also obtained an abrupt increase in alkalinity after 1.5 equivalents had been added, the  $pH$  of the solution rising to above the point ( $pH$  8.5-9.0) at which errors are introduced into the quinhydrone determination through the neutralisation of the hydroquinone. Indicator methods, in the case of

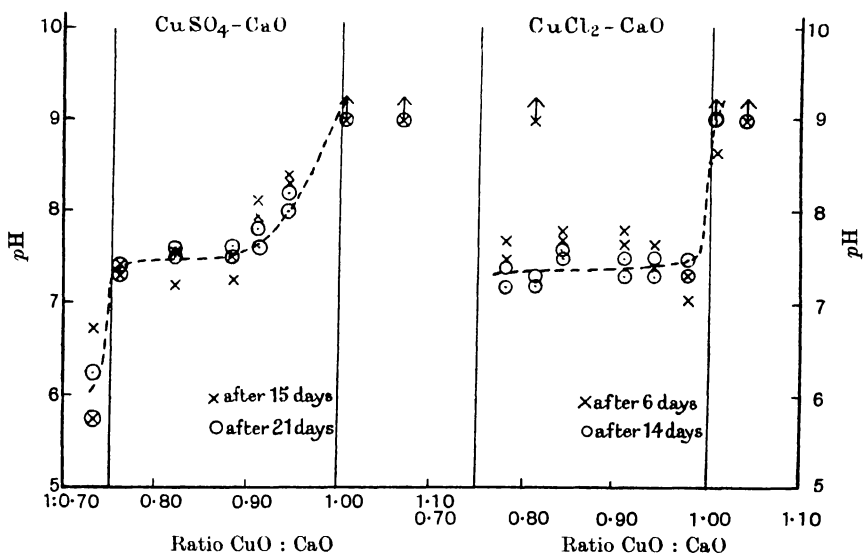


Fig. 4.

the calcium hydroxide reaction, led Pickering to consider the solution permanently alkaline at the ratio  $CuO : CaO = 10 : 9$ ; Sicard, 10 : 10; Wöber, 10 : 8.

Determinations of the hydrogen-ion concentration of the solution by means of the quinhydrone electrode by the method described on p. 115, are represented by the two graphs in Fig. 4. In the sulphate curve, the sudden increase in  $pH$  at the ratio  $CuO : CaO = 1 : 0.75$  indicates the formation of the  $4CuO \cdot SO_3$  basic sulphate and a less steep rise after 0.9 equivalents of lime water had been added does not give definite alkalinity until nearly 1 equivalent of calcium hydroxide is present. In the chloride curve the inflection at the ratio  $CuO : CaO = 1 : 1$  is

most marked and gives conclusive proof that the interaction of cupric chloride and calcium hydroxide involves equivalent amounts of the two reagents. In the same curve it will be noticed that, at the ratio  $\text{CuO} : \text{CaO} = 1 : 0.8$ , when only a slight excess of lime is present, its interaction with the basic chloride proceeds slowly and equilibrium was not reached by the sixth day. This phenomenon was observed also in Experiment E, Series 2, when the *pH* of the solutions shows a decrease with the amount of lime water added. Equilibrium is attained more rapidly when a larger excess of lime is present.

The conclusion that permanent alkalinity is produced only when amounts of lime in excess of the ratio  $\text{CuO} : \text{CaO} = 1 : 1$  are added is confirmed by the colour changes given with B.D.H. Universal indicator, recorded in Experiments A and C. In the cupric sulphate trials, two points of inflection on the neutralisation curve are clearly defined, the first, between the ratios  $\text{CuO} : \text{CaO} = 1 : 0.707$  and  $1 : 0.816$  ( $1 : 0.799$  in Experiment A, Series 3), corresponding to the complete conversion of the copper sulphate to the  $4\text{CuO} \cdot \text{SO}_3$  basic sulphate; the second, between  $\text{CuO} : \text{CaO}$  ratio of approximately  $1 : 1$ , indicating the complete decomposition of the  $4 : 1$  sulphate. Definite alkalinity, shown by the violet colour, only occurs with molar ratios  $\text{CuO} : \text{CaO}$  of over  $1 : 1$ . In the cupric chloride reaction there is a similar colour change extending over the ratios  $\text{CuO} : \text{CaO} = 1 : 0.996$  and  $1 : 1.087$ . The more alkaline colour at the ratio  $\text{CuO} : \text{CaO} = 1 : 0.808$  is further evidence of the slowness of equilibrium attainment when small amounts of lime water are added to the basic chloride suspension.

In the case of the cupric chloride reaction, the point of inflection on the neutralisation curve at the ratio  $\text{CuO} : \text{CaO} = 1 : 1$ , and the re-appearance of the whole of the precipitated chloride in solution, indicate that the reaction may be represented by the empirical equation:



The formation of the nigger-brown hydrated oxide is typical of the interaction of cupric salts with strong alkalis. A gelatinous blue precipitate is produced which, on standing, blackens more or less rapidly according to the experimental conditions, a change which is usually regarded as a process of dehydration. The blue precipitate which is initially produced in calcium hydroxide-cupric chloride reaction and which rapidly blackens, is of the same nature. Although it does not appear, from the literature, whether the blue precipitate is to be regarded as cupric hydroxide or as an hydrated oxide, it will be called for convenience

the hydroxide, an assumption which will not affect the subsequent argument.

The general similarity between the chloride and sulphate curves in Fig. 4 suggests that the action of calcium hydroxide upon the  $4\text{CuO} \cdot \text{SO}_3$  basic sulphate likewise results in the formation of cupric hydroxide. That the final insoluble product of each reaction is identical is indicated by the fact that the hydrogen-ion concentrations shown by the horizontal portions of the curves are similar; the  $[\text{OH}']$  components of the solubility products of the two precipitates are therefore equal.

The examination of the hydrogen-ion concentration curve indicates that the non-appearance of the total sulphate radicle in solution is due to adsorption. To postulate the existence of a basic copper-calcium sulphate to explain the change of direction of the sulphate in solution curve at the ratio  $\text{CuO} : \text{CaO} = 1 : 0.9$  becomes unnecessary and it follows that the precipitate, obtained when equimolecular amounts of copper sulphate and lime water are mixed, is cupric hydroxide upon which calcium sulphate is strongly adsorbed. The initial product of the reaction is the  $4\text{CuO} \cdot \text{SO}_3$  basic sulphate which, owing to its slight hydrolysis, slowly reacts with the excess calcium hydroxide to give cupric hydroxide. It thus becomes necessary to explain the fact that the cupric hydroxide so produced does not undergo the dehydration which occurs with the cupric chloride-lime water precipitate.

### (3) *The dehydration of the precipitate.*

Dehydration to form the brownish black cupric oxide occurred with the precipitate produced by the interaction of cupric chloride and amounts of lime water within the molar ratios  $\text{CuO} : \text{CaO} = 1 : 0.863$  and  $1 : 1.062$  in Experiment C,  $1 : 0.849$  and  $1 : 1.044$  in Experiment D, Series 2. The corresponding mixtures of copper sulphate and lime water showed no sign of darkening. Pickering (19), however, observed that on boiling the precipitate produced from copper sulphate and lime water at ratios of  $\text{CuO} : \text{CaO}$  of between  $1 : 0.833$  and  $1 : 1.15$  decomposed, a fact which he considered evidence of the formation of an unstable  $5\text{CuO} \cdot \text{SO}_3$  basic sulphate. That dehydration does occur, though at  $100^\circ \text{C}.$ , in the sulphate reaction at the same  $\text{CuO} : \text{CaO}$  ratios as with the chloride reaction might also indicate the similarity of the two reactions. The chief difference in chemical character between the two precipitates is that the Bordeaux mixture precipitate contains much adsorbed calcium sulphate and it seems probable that this factor is responsible for the retention of the blue colour by the precipitate at ordinary temperatures. Retardation of the

dehydration of cupric hydroxide in the presence of sulphate ions was observed by Finch<sup>(12)</sup> and by Weiser<sup>(24)</sup>. Confirmatory evidence of the stabilising action of adsorbed sulphate was obtained in Experiment E. The addition of calcium sulphate to the cupric chloride-calcium hydroxide mixture has prevented the blackening of the precipitate observed in the absence of the sulphate.

It follows that, if the copper sulphate-lime water precipitates obtained with molar  $\text{CuO} : \text{CaO}$  ratios greater than  $1 : 0.75$  can be freed from adsorbed sulphate and from excess of lime, they should undergo dehydration. Trials were therefore undertaken to discover the effect of prolonged washing with water upon the various basic precipitates.

The action of water upon the basic sulphate and upon the precipitate of Bordeaux mixture does not appear to have previously been studied, though it has often been assumed that, for the purposes of analysis, these materials can be freed from soluble impurities by washing with carbon dioxide-free water. In the case of the copper sulphate-calcium hydroxide precipitation both Sicard<sup>(22)</sup> and Wöber<sup>(26)</sup> washed the  $4\text{CuO} \cdot \text{SO}_3$  sulphate prior to analysis. Young and Stearn<sup>(27)</sup>, Bell and Murphy<sup>(1)</sup> and Nelson<sup>(17)</sup> likewise washed the basic sulphate produced by the precipitation of copper sulphate with sodium hydroxide. Nelson added sodium hydroxide in excess of 1.5 equivalents yet washed the precipitate free from soluble sulphate upon the tacit assumption that the precipitate was not decomposed by water. Hooker<sup>(14)</sup>, on the other hand, stated that the action of water upon the basic sulphate produced by adding sodium hydroxide insufficient in amount to cause complete precipitation of copper from copper sulphate resulted in the formation of a blue hydroxide. In Experiment F, Series 1, continued washing of the  $4\text{CuO} \cdot \text{SO}_3$  precipitate obtained by adding less than 0.75 equivalents of calcium hydroxide to copper sulphate, showed that this salt is stable, confirming Fowles' <sup>(13)</sup> statement.

With the more alkaline Bordeaux mixtures there are a few isolated references to the decomposition of the precipitate on washing. Wöber mentioned that prolonged washing with carbon dioxide-free water of the precipitate obtained with  $\text{CuO} : \text{CaO}$  ratios greater than  $1 : 1$ , a complete removal of calcium compounds was not possible without decomposition of the precipitate. McCallan<sup>(16)</sup> also referred to the disorganisation of the precipitate of Bordeaux mixture when washed with large quantities of water. An examination of the effects of washing with carbon dioxide-free water, the precipitate obtained by the addition of more than 0.75 equivalents of calcium hydroxide to copper sulphate solutions is described in Experiment F, Series 2 (p. 116). In such cases complete or partial

dehydration of the precipitate occurred. Even with the "equal lime" Bordeaux mixture (Experiment F, Series 3) dehydration of the precipitate was produced by continued washing with water.

This result has an important practical bearing for, when sprayed upon foliage, the Bordeaux mixture precipitate will be subjected to continued washing by rain and dew. That, under these conditions, dehydration does not occur is shown by the retention of the blue colour of the spray deposit. The precipitate, in drying on the leaf, has therefore undergone some physical or chemical change whereby dehydration is prevented. That the physical change brought about by drying the precipitate is unable to prevent blackening is shown in Experiment G. Various Bordeaux mixtures were, in this experiment, allowed to stand in a Young's rod and disc fractional distillation tube arranged in a horizontal position. The supernatant liquid was drawn off and the precipitate dried by the continued passage of air through the tube. An adherent Bordeaux deposit was thus obtained which could be washed with water. In those trials in which atmospheric carbon dioxide was excluded blackening of the precipitate occurred. But when no precaution was taken to exclude carbon dioxide a Bordeaux deposit was obtained which was unchanged in colour by continued washing.

It must be concluded therefore that the retention of the blue colour by the Bordeaux precipitate upon foliage is due to chemical changes in which carbon dioxide is involved. The investigation of the action of carbon dioxide upon the  $4\text{CuO} \cdot \text{SO}_3$  basic sulphate and upon copper hydroxide which is formed from this compound by the action of lime will, it is hoped, throw light upon the nature of this reaction. A discussion of the corollaries of this conclusion must be held over until the nature of the deposit formed after spraying has been elucidated but there are two points which may be dealt with briefly at this stage.

That the Bordeaux deposit is formed *in situ* upon the leaf by the action, primarily, of carbon dioxide upon the copper sulphate-lime precipitate accounts for the excellent adhesiveness of the deposit. It has frequently been observed (see Boyd(3)) that a common cause of failure of substitutes for Bordeaux mixture is the rapid loss, from the foliage, of the protective copper-containing film. It is probable that in many cases this lack of adhesiveness is associated more with the chemical inertness of the substitute towards carbon dioxide, leaf excretions and other reactants which may come into play upon the foliage, than with the physical character of the substitute. In the case of Bordeaux mixture, many investigators have stressed the importance of the study of the physical nature of the precipitate. De Ong and Root(10) used the rate



of settling, whilst Butler (8) and others have employed the voluminousness of the precipitate as criteria for the adhesiveness of the precipitate. The value of such tests as indicators of the fungicidal efficiency of the spray may be profoundly affected by the susceptibility of the precipitate to these chemical changes which occur after spraying.

#### EXPERIMENTAL.

*Exp. A.* Definite volumes of lime water were added rapidly from an automatic pipette to flasks containing 25 ml. 4 per cent. A.R. copper sulphate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) and sufficient boiled-out distilled water to make the final volume up to 250 ml. The flasks, closed with a rubber stopper carrying a soda-lime tube to exclude atmospheric carbon dioxide, were placed in a thermostat at  $21.5^\circ \text{C}$ . In a number of the trials carbon dioxide was excluded by passing through the flasks carbon dioxide-free nitrogen, a pressure slightly greater than atmospheric being maintained within the flasks throughout the trial. The flasks were shaken from time to time and after the lapse of a definite period—seven or twenty-eight days—were removed from the thermostat, allowed to stand overnight and the contents sampled.

For analysis 100 ml. of the supernatant liquid, withdrawn by means of a pipette, were used for the estimation of calcium in solution. The contents of the flask were then poured on a rapid filter and, without delay, 100 ml. of the filtrate were taken for the estimation of sulphate and copper content. 20 ml. of the filtrate were also used for an estimate of hydrogen-ion concentration with the B.D.H. Universal indicator. The precipitate, with the remaining 30 ml. filtrate, was dissolved in the minimum of hydrochloric acid and the content of calcium, copper and sulphate determined.

Table I.

*Series 1. Mixtures contained 0.3225 gm.  $\text{SO}_3$  per 250 ml., and were left in thermostat for 7 days.*

Lime water added gave molar ratio $\text{CuO} : \text{CaO}$	$\text{SO}_3$ in solution		Colour with B.D.H. Universal indicator	In precipitate		
	gm.	% of total		$\text{SO}_3$ gm.	$\text{CuO}$ gm.	$\text{CaO}$ gm.
1 : 0.133	0.3077	95.4	Orange	0.0130	0.0577	Nil
1 : 0.266	0.2938	91.1	Orange	0.0303	—	—
1 : 0.417	0.2780	86.2	Orange	0.0427	0.1827	—
1 : 0.532	0.2636	81.7	Orange	0.0602	0.2295	Nil
1 : 0.696	0.2502	77.6	Orange	0.0746	—	—
1 : 0.706	0.2433	75.4	Orange	0.0773	0.2997	0.0011
1 : 0.899	0.2557	79.3	Greenish yellow	0.0693	0.3205	0.0200
1 : 0.963	0.2574	79.8	Greenish yellow	0.0643	0.3205	0.0309
1 : 1.064	0.2557	79.3	Violet	0.0674	0.3205	0.0530
1 : 1.091	0.2575	79.8	Violet	0.0648	0.3205	0.0527
1 : 1.155	0.2565	79.5	Violet	0.0637	0.3205	0.0608

Table II.

*Series 2. Mixtures contained 0.3225 gm. SO<sub>3</sub> per 250 ml., and were left in thermostat for 28 days.*

Lime water added gave molar ratio CuO : CaO	SO <sub>3</sub> in solution		Colour with B.D.H. Universal indicator	In precipitate				Molar ratio CuO : SO <sub>3</sub>
	gm.	% of total		SO <sub>3</sub> gm.	CuO gm.	CaO gm.		
1 : 0.133	0.3040	94.0	Orange	0.0158	0.0582	Nil	3.7 : 1	
1 : 0.266	0.2912	90.3	Orange	0.0304	—	—	—	
1 : 0.417	0.2752	85.3	Orange	0.0457	0.1814	—	4.0 : 1	
1 : 0.532	0.2636	81.7	Orange	0.0603	0.2315	Nil	3.9 : 1	
1 : 0.545	0.2617	81.1	Orange	0.0591	0.2362	Nil	4.0 : 1	
1 : 0.598	0.2520	78.0	—	0.0681	0.2657	Nil	3.9 : 1	
1 : 0.696	0.2513	77.9	Orange	0.0708	—	—	—	
1 : 0.707	0.2429	75.3	Orange	0.0783	0.3095	0.0001	4.0 : 1	
1 : 0.816	0.2511	77.8	Yellowish green	0.0734	0.3221	0.0082	—	
1 : 0.870	0.2599	80.6	Greenish yellow	0.0623	0.3212	0.0184	—	
1 : 0.925	0.2615	81.1	—	0.0614	0.3205	0.0246	—	
1 : 1.034	0.2589	80.3	Bluish green	0.0608	0.3198	0.0499	—	
1 : 1.064	0.2571	79.7	Violet	0.0648	0.3175	—	—	
1 : 1.142	0.2586	80.2	Violet	0.0647	0.3205	0.0550	—	

Table III.

*Series 3. The final volume of the copper sulphate-lime water mixture was adjusted to 450 ml., 25 ml. 3 per cent. copper sulphate being used. The flasks were left for 28 days before sampling for analysis.*

Lime water added gave molar ratio CuO : CaO	In solution		Colour with B.D.H. Universal indicator
	gm. SO <sub>3</sub>	% of total	
1 : 0.799	0.1875	76.9	Greenish yellow
1 : 0.879	0.1945	79.7	Greenish yellow
1 : 0.959	0.1993	81.6	Greenish yellow
1 : 1.039	0.1971	80.7	Greenish yellow
1 : 1.118	0.1966	80.6	Blue
1 : 1.198	0.1922	78.7	Violet

A final check upon the accuracy of the determinations was obtained by calculating from the analytical figures the total amounts of copper, calcium and sulphate radicle present. Unless this total agreed within 1 per cent. to the amounts of copper sulphate solution and lime water initially used, the figures were discarded.

*Exp. B.* 1 gm. amounts of calcium sulphate (B.D.H. pure precipitated) were weighed into 300 ml. flasks, various amounts of saturated lime water were added and the volume adjusted to 250 ml. with boiled-out distilled water. The flasks were closed with a rubber bung carrying a soda-lime tube to exclude carbon dioxide and were left, with intermittent

shaking for 5 weeks in a thermostat at 21.5° C. Aliquots of the clear supernatant liquid were then withdrawn for analysis. Calculation of the quantities of sulphate and of calcium per 100 ml. solution gave the following figures:

SO <sub>3</sub> :	0.1227	0.1175	0.1142	0.1110 gm.
CaO:	0.0845	0.0911	0.0978	0.1040 gm.

The figures determined by Cameron and Bell(8) who studied the system CaSO<sub>4</sub> - CaO - H<sub>2</sub>O at 25° C. give the following figures:

SO <sub>3</sub> :	0.1250	0.1194	0.1090 gm. per 100 ml.
CaO:	0.0876	0.0898	0.1112 gm. per 100 ml.

*Exp. C.* For the investigation of the interaction of cupric chloride and lime water, an experimental procedure similar to that employed in *Exp. A* was used, an equivalent solution of cupric chloride (27.5 gm. A.R. CuCl<sub>2</sub> · 2H<sub>2</sub>O per litre) replacing the copper sulphate solution. The flasks were placed in a thermostat at 21.5° C. for one week before sampling.

Table IV.

*Mixtures contained 0.2883 gm. CuO per 250 ml.*

Lime water added gave molar ratio CuO : CaO	Cl in solution		Colour with B.D.H. Universal indicator	Colour of precipitate
	gm.	% of total		
1 : 0.797	0.2214	76.8	Orange	Greenish blue
1 : 0.808	0.2246	77.9	Green	Greenish blue
1 : 0.863	0.2278	79.0	Greenish yellow	Nigger brown
1 : 0.896	0.2439	84.6	—	Nigger brown
1 : 0.930	0.2461	85.3	Greenish yellow	Nigger brown
1 : 0.959	0.2608	90.4	—	Trace of black
1 : 0.996	0.2621	90.9	Bluish green	Bluish grey
1 : 1.062	0.2802	97.2	Bluish green	Bluish grey
1 : 1.087	0.2866	99.4	Violet	Blue
1 : 1.119	0.2866	99.4	Violet	Blue
1 : 1.129	0.2866	99.4	—	—
1 : 1.152	0.2877	99.8	—	—
1 : 1.196	0.2856	99.0	Violet	Blue
1 : 1.262	0.2866	99.4	—	—

The estimation of chloride in solution when amounts of lime water less than the molar ratio CuO : CaO = 1 : 0.75 were added was frustrated by the nature of the precipitate produced. An opalescent greenish blue colloidal solution was formed which made it impossible to remove the basic chloride by filtration or by sedimentation. It has been observed by Britton(8) that the production of colloidal solutions of basic salts is a characteristic tendency of chlorides.

*Exp. D, Series 1.* Mixtures containing known amounts of copper sulphate and calcium hydroxide were prepared, as in *Exp. A*, by the

addition of definite volumes of lime water to 50 ml. 4 per cent. copper sulphate diluted to give a final volume of 500 ml. The solutions were protected from atmospheric carbon dioxide by covering with a layer of medicinal paraffin.

To determine the hydrogen-ion concentration of the supernatant liquid, about 5 ml. were withdrawn with a pipette and added to a small cup-shaped separating funnel containing solid quinhydrone and sufficient medicinal paraffin to give a protective layer over the solution. The presence of medicinal paraffin did not interfere with the determination except by reducing the sensitiveness of the platinum electrode—a result probably of the continued ignition of the trace of paraffin left on the electrode after washing. As the null point reading was not always sharply defined, a wide interpretation was given to the millivolt reading obtained. The ability of the paraffin layer to exclude carbon dioxide was shown by a preliminary trial in which a dilute solution of calcium sulphate, made slightly alkaline with 4 drops of lime water, was allowed to stand under paraffin. The initial pH of the solution was 8.75–8.85 and after standing 27 days the figure was reduced only to pH 8.55–8.60.

Table V.

*Series 1. Copper sulphate-lime water.*

Lime water added gave molar ratio CuO : CaO	pH of supernatant solution after standing			
	5 days	7 days	15 days	21 days
1 : 0.735	5.6–6.0	5.6–6.0	5.75–6.75	5.75–6.25
1 : 0.765	6.5–6.8	6.9–7.2	7.3–7.4	7.3–7.4
1 : 0.826	7.0–7.2	7.5–7.6	7.2–7.55	7.5–7.6
1 : 0.887	7.0–7.2	7.6–7.7	7.25–7.5	7.5–7.6
1 : 0.918	7.8–7.9	8.0–8.1	7.95–8.15	7.6–7.8
1 : 0.949	8.7	8.4	8.3–8.4	8.0–8.2
1 : 1.010	>9.0	>9.0	>9.0	>9.0
1 : 1.071	>9.0	>9.0	>9.0	>9.0

*Series 2. Copper chloride-lime water.*

Lime water added gave molar ratio CuO : CaO	Colour of precipitate after 2 days	pH of supernatant solution after standing	
		6 days	14 days
1 : 0.783	Greenish blue	7.5–7.7	7.2–7.4
1 : 0.816	Greenish blue	>9.0	7.2–7.3
1 : 0.849	Nigger brown	7.6–7.8	7.5–7.7
1 : 0.914	Nigger brown	7.65–7.8	7.3–7.5
1 : 0.947	Nigger brown	7.4–7.65	7.3–7.5
1 : 0.980	Nigger brown	7.05–7.3	7.3–7.5
1 : 1.012	Nigger brown	8.65	>9.0
1 : 1.044	Blackish purple	>9.0	>9.0
1 : 1.110	Blue	>9.0	>9.0

*Exp. E.* A series of six cupric chloride-lime water mixtures was made up, in three of the six flasks saturated calcium sulphate solution replaced the water required to complete the volume to 250 ml. Since the retention of the blue colour at ratios  $\text{CuO} : \text{CaO}$  of over 1 : 1 is to be attributed to the alkalinity of the mixture, the hydrogen-ion concentration of the supernatant solution was determined.

Table VI.

Lime water added gave molar ratio $\text{CuO} : \text{CaO}$	gm. $\text{SO}_3$ pre- sent	Hydrogen-ion concentration after		Colour of precipitate after		
		6 days	18 days	1 day	6 days	18 days
1 : 0.849	Nil	8.1-8.2	7.5-7.7	Greenish blue	Blackish blue	Black, some bluish slate
1 : 0.849	0.110	7.2-7.5	7.3-7.5	Blue, with tinge of greenish blue	Blue	Blue
1 : 0.914	Nil	7.9-8.1	7.3-7.5	Greyish black	Nigger brown, some blackish blue	Nigger brown
1 : 0.914	0.098	7.2-7.5	7.4-7.6	Blue	Blue	Blue
1 : 0.979	Nil	7.0-7.4	7.3-7.5	Almost black	Nigger brown	Nigger brown
1 : 0.979	0.087	7.2-7.4	7.3-7.5	Blue	Blue	Blue

*Exp. F, Series 1.* Two litres of lime water were added to a solution containing 10.50 gm.  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , precautions being taken to exclude carbon dioxide. The bottle was closed with a stopper carrying two tubes, one reaching almost to the bottom of the bottle to enable the withdrawal of the supernatant liquid by means of a filter pump, the second permitting air free from carbon dioxide to enter. By connecting the second tube to the filter pump, a quantity of freshly-boiled distilled water equal in volume to the liquid removed was added through the first tube. Washings were repeated when the precipitate had settled. After five washings the amount of calcium sulphate in the wash water was too small for analysis. The amounts of calcium oxide and of copper sulphate used were known and by subtraction of the total amounts of calcium oxide, copper and sulphate radicle removed in the wash water, the molar ratio  $\text{CuO} : \text{SO}_3$  in the precipitate was calculated to be 4.05 : 1.

*Series 2.* Lime water was added to copper sulphate solutions prepared by diluting 50 ml. 4 per cent. copper sulphate with carbon dioxide-free water to amounts necessary to give a final volume of 450 ml. The mixtures were protected from atmospheric carbon dioxide by covering with a layer of medicinal paraffin. After standing 3 weeks the super-

natant solutions were withdrawn by syphoning and the volume restored by adding boiled distilled water. Every 12 hours the process was repeated, and, after the sixth washing, the mixtures were left for 48 hours. The colour changes of the precipitate are shown in Table VII.

Table VII.

Lime water added gave molar ratio CuO : CaO	Colour of the precipitate after		
	4 washings	5 washings	6 washings
1 : 0.735	Greenish blue	Greenish blue, partly colloidal	Greenish blue, partly colloidal
1 : 0.765	Blue, with greenish tint	Blue, with greenish tint	Blue
1 : 0.826	Blue	Blue	Bluish slate
1 : 0.887	Blue	Signs of blackening	Blackish blue
1 : 0.918	Signs of blackening	Blackish grey	Nigger brown
1 : 0.949	Definite blackening	Nigger brown	Nigger brown
1 : 1.010	Blue	Definite blackening	Blackish grey
1 : 1.071	Blue	Definite blackening	Blackish grey

*Series 3.* Bordeaux mixture (8 : 8 : 100) was prepared by the addition of 80 ml. 10 per cent. copper sulphate solution to 920 ml. water containing 12 gm. hydrated lime. The mixture was allowed to settle and the supernatant liquid withdrawn by suction. An amount of carbon dioxide-free water equal in volume to the solution withdrawn was added and the mixture shaken. After settling, the process was repeated. After seven such treatments the bluish mauve precipitate began to darken in colour and, after ten washings, it was nigger brown in colour. After further washings the precipitate became partly colloidal and washing by decantation was impossible.

*Exp. G.* To obtain a deposit of Bordeaux mixture dried in the absence of carbon dioxide, the precipitate was transferred by suction to a Young's rod and disc fractional distillation tube, containing 15 discs, of which the thermometer opening was closed by a waxed rubber bung. The precipitate was allowed to settle and the supernatant liquid withdrawn by gentle suction, the delivery tube being connected to a train of three U-tubes filled with calcium chloride and three of soda lime. Air thus dried and freed from carbon dioxide was drawn over the deposit until all moisture had been removed. In a second distillation tube, placed alongside the first, no precautions were taken to exclude carbon dioxide. The rate of flow of air through the two tubes was similar.

An "equal lime" Bordeaux mixture was prepared by the addition of 40 ml. 3 per cent. crystalline copper sulphate to 1 litre of lime water.

The precipitate was allowed to settle and transferred to the Young's tubes. After drying, the colour of the deposit in the carbon dioxide-free tube was a purer and deeper blue than that dried without removal of carbon dioxide. The calcium chloride tubes were then disconnected and the Young's tubes connected with flasks containing boiled-out distilled water, a slow stream of water being drawn over the Bordeaux deposit by means of a filter pump. After the passage of 4 litres of water the deposit in the tube protected from carbon dioxide began to blacken just below the entrance tube and after 12 litres had passed (24 hours) the whole of the deposit in the tube was definitely blackened. The deposit in the tube, unprotected from carbon dioxide during drying, was still blue in colour and was apparently unaffected.

Similar results were obtained with Bordeaux mixtures prepared by the addition of copper sulphate solution to lime water in amounts giving CuO : CaO ratios of 1 : 1 and 1 : 2.

#### SUMMARY.

1. The initial product of the interaction of cupric sulphate and calcium hydroxide solutions, at ordinary temperatures, is the  $4\text{CuO} \cdot \text{SO}_3$  basic sulphate.

2. With amounts of calcium hydroxide in excess of 0.75 equivalent, the  $4\text{CuO} \cdot \text{SO}_3$  salt is slowly decomposed with elimination of the sulphate radicle and the formation of a blue hydrated cupric oxide or cupric hydroxide, a product called, for convenience, the hydroxide.

3. The cupric hydroxide formed retains, by adsorption, part of the sulphate radicle. Direct evidence by analysis of the precipitate, which might be considered to indicate the formation of a basic copper-calcium complex, is therefore unreliable.

4. In the analogous interaction of calcium hydroxide with cupric chloride, the formation of cupric hydroxide in the presence of amounts of calcium hydroxide in excess of 0.75 equivalent, is indicated by the dehydration of the precipitate to form nigger brown hydrated cupric oxide, and by the definite point of inflection on the hydrogen-ion concentration curve which corresponds to equimolecular amounts of calcium hydroxide and cupric chloride.

5. The hydrogen-ion concentration curve for the cupric sulphate-calcium hydroxide reaction shows that permanent alkalinity is not reached until equimolecular amounts of calcium hydroxide have been added.

6. The cupric hydroxide, formed from the  $4\text{CuO} \cdot \text{SO}_3$  basic sulphate by the action of calcium hydroxide, is stabilised by adsorbed sulphate ions. If the adsorbed sulphate and excess calcium hydroxide be removed by continued washing with carbon dioxide-free water, the Bordeaux mixture precipitate undergoes dehydration to form the nigger brown hydrated cupric oxide.

7. Dehydration of the Bordeaux mixture precipitate when sprayed upon foliage, is therefore prevented by some chemical or physical change of the precipitate which occurs on drying.

8. Dehydration is not prevented by physical changes which occur on drying.

9. Carbon dioxide is a factor in the process whereby dehydration is prevented.

10. It is suggested that the formation *in situ* of the protective copper-containing deposit by the action, primarily, of carbon dioxide, accounts for the excellent adhesive properties of the Bordeaux mixture precipitate; that inertness towards the chemical changes which occur after spraying may be the cause of failure of some Bordeaux substitutes; that these changes may profoundly affect the value of sedimentation tests as criteria for the fungicidal efficiency of various Bordeaux mixtures.

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(Received March 12th, 1931.)

## REVIEWS.

*Die Tierischen Schädlinge des Ackerbaues.* Von SOFIE ROSTRUP und MATHIAS THOMSEN; ins Deutsche übertragen von Dr H. BREMER und Dr R. LANGENBUCH. Berlin: Verlagsbuchhandlung Paul Parey, 1931. Pp. 367. 236 text-figs. Price R.M. 18.

The original (Danish) edition of this book was published in 1900 by Miss Rostrup. It was founded on her own observations and experience, and little use was made of work carried out in other countries. The fourth edition, of which this is a German translation, has been entirely revised and brought up to date by Rostrup and Thomsen, and references to foreign work have been incorporated. The German translation was undertaken because no work of this kind was available in that language, and the pests of farm crops in Denmark and in Germany, particularly North Germany, are almost identical. A few additional sections have been added by the translators, in order to include pests which are important in Germany though not so in Denmark.

In the preface it is stated that the work is intended primarily for growers, agricultural students, and the like. Details of morphology, anatomy, and classification have therefore been reduced to a minimum and technical terms as far as possible avoided.

A general outline of the problem is given in the introduction. After an estimate of the amount of damage caused by invertebrate pests, the various methods of dealing with them are briefly discussed. Cultural methods, such as changing the rotation of crops, are those most frequently adopted in farming operations: except in the case of garden and market garden produce, direct chemical means can seldom be used owing to the cost. Biological control has so far proved of little significance, and the use of trap crops, which is so frequently recommended in theory, is of little practical use. The introduction concludes with short notes on the various insecticides in common use.

The arrangement of the main part of the book is straightforward, the pests being dealt with according to their Phyla, viz. Nematoda, Chaetopoda, Arthropoda and Mollusca. In the case of the Arthropoda, a simple key to the classes Crustacea, Myriapoda, Insecta and Arachnida, is given, and a further key to the Orders in the Insecta. Vertebrate pests are not included. After a short account of the Phylum in general, and in most cases of the Order and family, there is a detailed account of each pest. Particular attention is paid to a description of the characteristic damage caused, to the bionomics of the pest, and to the control measures recommended against it. In the majority of cases both the pest and the damage caused are adequately illustrated by drawings or photographs, most of which are original. Following the text there is a key for the recognition of the pests from the damage done to the host plants. The practical value of such a key, however, is doubtful and a simple list of the main crops with their pests, with cross references to the text, would possibly have been of more value. A bibliography of the more important general text-books, and of publications dealing with individual pests is given and there is an index of the pests described.

There are few criticisms that can be levelled against this book. The classification adopted is hardly up to date and the same applies to the nomenclature in some cases, but these are minor points in a book of this kind. There are, however, a number of omissions; no mention, for instance, being made of the Onion Fly (*Hylemyia antiqua*) which is a serious pest in parts of Germany. Glasshouse pests are scantily dealt with, no account being given of one of the most serious, *Trialeurodes vaporariorum*, and no control measures suggested for the Red Spider. Chemical sterilisation of the soil for *Heterodera radiculicola* in glasshouses is suggested, but steam sterilisation, which is the standard treatment, in this country at all events, is not mentioned. The addition of a general index would also be an improvement.

On the whole, however, the book is remarkably comprehensive and free from mistakes, and it will be of great assistance to agricultural zoologists in this country, both as a textbook for teaching and as a convenient reference book in which the standard control measures for the majority of farm pests can readily be found.

A. S. BUCKHURST.

*An Introduction to the Literature of Vertebrate Zoology based chiefly on the titles in the Blacker Library of Zoology, the Emma Shearer Wood Library of Ornithology, the Bibliotheca Osleriana and other Libraries of McGill University, Montreal.* Compiled and edited by CASEY A. WOOD, M.D., LL.D. London: Oxford University Press, 1931. 4to, pp. xix + 643. Col. frontispiece. Price, \$15 or £3. 3s.

This volume has been compiled to assist students of vertebrate zoology and its bibliography. About two-thirds of the works in the Catalogue are on the shelves of the Blacker Library. McGill University is fortunate in possessing this and the other notable collections of books mentioned in the title and in having an endowment adequate for the continuation of the zoological journals and for the yearly addition of the most important new publications on vertebrate zoology. One of the "other libraries" referred to in the title is the Gest Library of Chinese literature which consists of more than 50,000 volumes all of which, except a few modern works, were in the possession of Manchu princes, well known statesmen or bibliophiles. This important collection comprises works on almost all departments of Chinese civilisation.

"The reader is warned in advance that this work makes no claim to the (as yet) unfilled position of a complete study in English of the literature of vertebrate zoology"; it is modestly put forward "as a basis for an elementary thesis" on this literature as it is represented by independent publications in the McGill libraries.

The work is divided into three parts. Section A (pp. 1-149) is an introduction to the literature of vertebrates from the earliest times to 1930, arranged in nineteen chapters each of which deals concisely with some period (e.g. mediæval writers on zoology and their successors) or with some special subject (e.g. zoogeography, ecology and palæontology). Section B (pp. 150-172) consists of a short title-index of the literature arranged geographically and in chronological order so that the reader can quickly locate any ordinary treatise and many of the rarer works. In the catalogue which forms Section C (pp. 173-643) each work is listed with bibliographical data and in many cases with annotations.

The volume which is beautifully produced, has as frontispiece a reproduction in colour of an aquarelle attributed to Charles Collins (ca. 1736) of the Dodo from the original drawing in the Blacker Library.

J. H. ASHWORTH.

*Economic Biology for Students of Social Science. Part II, Animal and Vegetable Products.* By PHILIPPA C. ESDAILE, D.Sc. University of London Press, 1931. Pp. xv + 231. 10s. 6d. net.

Part I of this work dealing with "Harmful and Useful Animals" received notice in this *Journal*, xiv, 4, 1927: Part II deals with "Animal and Vegetable Products" connected with the study of "Household and Social Science." The chapter headings are as follows: I, Leather, parchment and vellum; II, Fur; III, Hair and wool; IV, Horn, ivory and whalebone; V, Bone; VI, Glue, isinglass and catgut; VII, Fats, waxes and oils; VIII, Milk and milk products; IX, Dyes; X, Oils and fats; XI, Gums and resins; XII, Fibres; XIII, Starches, sugars and cellulose; XIV, Trees, timber

and tree products; XV, Beverages; XVI, Vinegar; XVII, Flavourings, spices, herbs and condiments.

The field covered is vast and it must have been difficult to decide what to include, *e.g.* edible and purely ornamental products are mostly omitted; brandy, rum and gin are included whereas benedictine, kirsch, saki and other spirituous liquors are omitted; leather is included, whereas all the modern reptilian and avian substitutes are left out; the only mention of feathers is that a preparation of turkey quills is a satisfactory substitute for whalebone, and so forth.

The book is clearly written, but there are too many "It is said that," "Statistics tell us," "So observers relate" and, throughout, there is considerable naïvety, *e.g.* "How has this come about? The story is not known. Nature, as mentioned above, has covered up her tracks." As numerous products are mentioned the treatment of individual items is necessarily brief and, in consequence, the book a little resembles an annotated catalogue. In order to make it more interesting, historical allusions are introduced, but these tend to become a little tedious and even social science students must feel tantalised and rebellious at the complete absence of detail when their appetites have been whetted by (p. 148) "the most gruesome torture that could only end in death."

The book is well printed and bound, contains no misprints, is illustrated by 96 text-figures and 11 tables and contains a glossary and an index. There are no references or suggestions for further study.

WILLIAM B. BRIERLEY.



TEMPERATURE AND HUMIDITY IN RELATION  
TO PROBLEMS OF INSECT CONTROL<sup>1</sup>

By A. D. IMMS, F.R.S.

*(Reader in Entomology, University of Cambridge.)**(With 2 Text-figures.)*

THE relations of temperature and humidity to insect control present problems of both theoretical and practical significance. Since these problems are manifold and complex, the present discourse is limited to a consideration of certain aspects only, and more especially in regard to recent developments in our knowledge. There is little justification to deal with the subject *in extenso*, since it has been admirably reviewed by Uvarov in a comprehensive memoir entitled *Insects and Climate*, published in 1931.

TEMPERATURE<sup>2</sup>.

*Constant temperatures.* If a number of individuals of a species, in the same phase of development, are divided into batches and each batch subjected to a different constant temperature, and the effects of these temperatures be evaluated, certain definite conclusions may be drawn. Given that the experiment has been conducted on a sufficiently ample scale, and the temperatures are plotted as abscissae, and the reciprocals of the factor chosen as ordinates, the resulting graph will approximate to a straight line in part of its course. This has been shown to be true whether the factor chosen be, for example, duration of development, CO<sub>2</sub> production, rate of movement, or the number of eye facets in *Drosophila*. Such a graph is a velocity chart which shows the rate of increase or acceleration, of the factor concerned, with rising temperature.

*Heat.* If the experiment has been carried sufficiently far into the zone of higher temperatures the graph departs from the straight line—in other words, a heat retardation becomes evident. The temperature at which vital activities begin to slow down depends upon various causes and, for many insects, it seems to be in the neighbourhood of 35°. In the case of growth, Peairs' experiments led to the conclusion that it is

<sup>1</sup> Presidential Address read before the Association of Economic Biologists on February 26th, 1932.

<sup>2</sup> Unless otherwise stated, temperatures are given in ° C.

rarely completed at 40°. The thermal death-point cannot be arbitrarily stated—much depends upon the duration at a given temperature and the gradations through which such a temperature is attained. The subject is one of great practical importance, since the method of heat sterilisation, in relation to insects affecting stored products, has come to the fore in recent years. In order to obtain results of practical value insects have been subjected to conditions that induce a lethal effect in the shortest time with the minimum expenditure of heat. There is a fairly general agreement that a temperature of 49–51.6° maintained for 12 hours is sufficient to destroy most insect life affecting stored grain and flour mills. Practical conclusions of this kind do not usually give actual thermal death-points, since much of the heat energy is absorbed in bringing up the stored material to a temperature that is lethal to the contained insects. According to Dendy and Elkington (1920), 5 min. duration at 62.8° will produce the same result as the longer duration at lower temperature just mentioned. Critical observations on high temperature gradients are few, and comparatively little is known with regard to the shape and trend of the constant temperature curve in this region, beyond the fact that it is relatively steep. Some of the most complete records are those of Shelford with reference to the codling moth (*Cydia pomonella*). He showed that, in so far as the pupal instar is concerned, the fall due to high temperatures is steeper than the rise on the opposite side of the curve. For the most part the temperature grades used have been large and much interpolation has been resorted to in constructing such curves. Laboratory experiments of this kind find their obvious practical application in problems of heat sterilisation of stored products.

The influence of heat on the duration of life has been shown, in a general way, to exercise a shortening effect in proportion to the rise in temperature. The most exact study of this phenomenon is found in the work of Alpatov and Pearl (1929) on *Drosophila*. Their experiments showed that, in this insect, length of life is doubled in the 10° of temperature reduction between 28 and 18°, which were the temperatures used. Females proved to be longer lived than males, and individuals of both sexes were used in the double series of experiments. In one series they were reared from the egg at 18° and in the other series at 28°. The adult flies were then tested as regards their viability at temperatures of 18, 25 and 28° and the results are shown in Fig. 1. It will be noted that the survival of individuals reared at 18° was marked longer than those at 28°. Alpatov and Pearl's conclusion that, at the higher tem-

peratures, the increased rate of energy expenditure during growth and imaginal life shortens survival, is what would be expected.

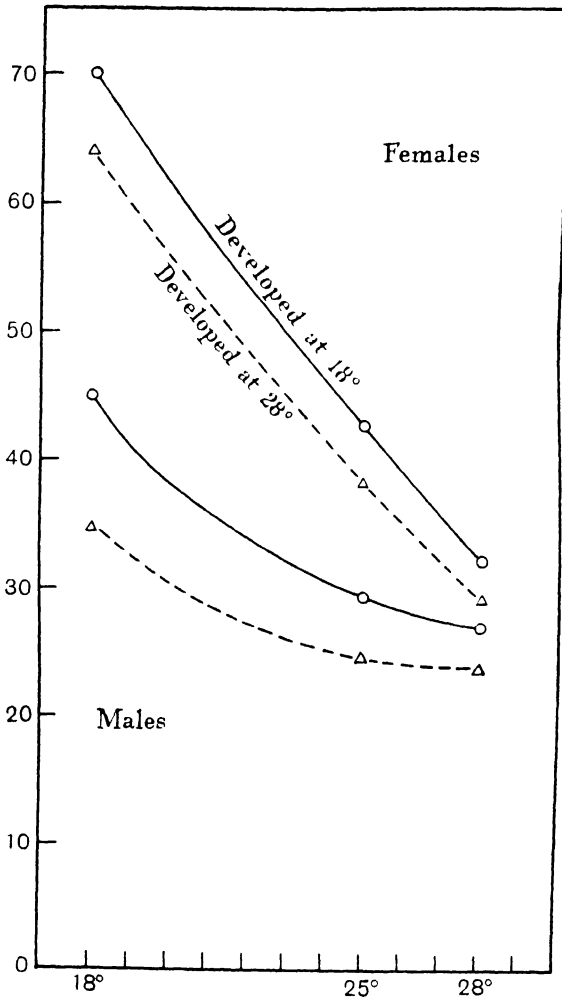


Fig. 1. Average duration of life in days: males (two lower curves) and females (upper curves) of *Drosophila*. Black lines indicate development at 18° and broken lines at 28°. (Adapted from Alpatov and Pearl, 1929.)

Of more direct interest to the applied entomologist is the influence of temperature on reproduction and fecundity. Here, however, evidence is both scanty and conflicting. Different groups of insects differ greatly as regards their physiology of reproduction. Higher temperatures may



accelerate the development of the germ cells but, on the other hand, as we have seen, they shorten the life of the individual. Pospelov (1911) showed that in certain Lepidoptera the ovaries only mature towards the end of the pupal stage and, when general development is accelerated by high temperatures, the adults emerge before they are sexually mature and fecundity is low. In more recent work of Titschak (1925), with reference to the clothes moth, it was found the females lived for 19 days at 20° and laid an average of 94.9 eggs apiece, while at 30° they lived only 6 days and laid an average of 79 eggs. The actual physiological causes involved are uncertain, and histological investigations were not carried out as in the case of Pospelov's work. The decrease in the number of eggs deposited appears, however, to be attributable, at least in part, to direct shortening of the life of the individual.

*Cold.* With regard to the lower end of the constant temperature curve all biological workers are familiar with the fact that there occurs a point on this curve just above which development begins to be measurable. This point is termed the threshold of development. The expression "physiological zero" is also used but is misleading. The physiological zero is a point below the threshold at which metabolic processes cease. The determination of the threshold of development for any given stage is laborious and by no means easy. It has to be remembered that just above this point so small an amount of development is going on that it requires a lengthy period of time to register the fact that any change has taken place at all. Further, in order to determine the shape of the velocity curve at this end a number of observations at short temperature intervals is necessary, which is a lengthy proceeding in view of the small amount of growth that takes place. Many investigators, in view of this difficulty, have assumed a theoretical threshold by taking it as being that point at which the velocity curve, when sufficiently prolonged, intersects the temperature line. In fact, all that is necessary for this method is to know accurately the duration of a given stage at two different constant temperatures lying within the straight line limits of the velocity curve. By producing the line joining these two points the theoretical threshold or  $\alpha$  point is obtained. Where actual determinations have been made it has generally been found that the velocity curve deviates from the straight line near its lower end, indicating an acceleration of development. In other words, development begins at a point lower in the temperature scale than that of the intersection of the velocity curve with temperature axis. In the extensive experiments of Peairs (1927) with eggs, larvae, and pupae of various insects the actual and

theoretical thresholds coincided, and I am not aware that any other observer has obtained consistently straight velocity curves.

For general phenological work, involving temperature summation, an arbitrary threshold has to be taken as the starting-point for these computations. Such summation takes into account the accumulation of day degree units above the assumed threshold. In a temperate climate such as our own a threshold of  $5.5^{\circ}$  ( $42^{\circ}$  F.) has been adopted for general purposes. The actual threshold has been ascertained for very few insects, but, from what is already known, we have to realise that this arbitrary threshold is too wide of the mark when a close approximation to accuracy is needed. Two examples will make this clear. Blunck, in Germany, found that for the eggs of the water beetle (*Dytiscus*) the threshold of development lies near  $0^{\circ}$ , while for the different larval instars it is between  $3$  and  $3.8^{\circ}$ . Shelford in America ascertained that in the codling moth (*Cydia pomonella*) the threshold varies according to the generation of the insect and to other factors. For the eggs he determined that it lay between  $6.5$  and  $9.5^{\circ}$ , and for the active non-hibernating larva, between  $6.1$  and  $8.8^{\circ}$ . It will be obvious, therefore, how wide the error will be when temperature summation is based upon a fixed arbitrary threshold. This error would be largely circumvented if constant temperature studies were made in the laboratory so as to allow of the determination of the  $\alpha$  point, for at any rate the more important pests. The determination of actual thresholds can hardly be hoped for and it is, in itself, a matter of more especially academic interest.

The relation of the threshold of development, and likewise the  $\alpha$  point, in host and parasites seem likely to have a definite bearing in connection with problems of biological control. Thus, in the well-known instance of the grain aphid (*Toxoptera graminum*) and its parasite (*Lysiphlebus tritici*), Shelford determined the curves of their development on the basis of the experimental data of Headlee and others. It appears that the threshold for the aphid is near  $0^{\circ}$ , while that for its parasite is  $2.2^{\circ}$ . It will be obvious that a temperature of  $1^{\circ}$  would arrest all development of the parasite while that of the host would go ahead. This, coupled with the fact that heat retardation commences to affect the parasite at a temperature of about  $5^{\circ}$  below that under which the host becomes susceptible, seems to suggest that small differences in reaction to critical temperatures may be important factors in the biological control of a species. In the present instance the aphid is not effectively controlled by its parasite. This same problem is being investigated by Miss M. G. Evans in the Zoological Laboratory at Cambridge with regard to two

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Tachinid parasites of *Dysdercus* and the *Digonochaeta* parasite of the common earwig. The *Dysdercus* suffers parasitisation in the field up to 80 per cent. of its individuals. Its  $\alpha$  point is  $47.2^{\circ}$  F. (being a tropical insect) while for its parasites this point is  $42.4^{\circ}$  F. and  $40.8^{\circ}$  F. respectively. Her observations indicate that development of the host is normally behind that of its parasites and a fall in temperature below  $47^{\circ}$  would inhibit the host's growth without affecting the parasites. The advantage in favour of the latter becomes further emphasised and egg-laying on the part of the host may become totally prevented. In the case of the earwig the reverse obtains. The influence of the parasite is negligible and, in the field, the proportion of hosts attacked is usually much below 12 per cent. The  $\alpha$  point for the host is definitely below that of the parasite and, during cold weather, the latter would receive a check to development while at the same temperature its host would remain unaffected. While no generalisations can be drawn from this line of inquiry at present, it appears to open up a suggestive field for investigators.

When insects are subjected to temperatures that descend below the threshold of development a point is reached where cold becomes fatal. This point varies enormously in different species, but is lowest in those which hibernate and is also dependent on several factors. Certain overwintering species are well capable of withstanding a temperature of  $-50^{\circ}$ . Remarkable as this fact is it seems to fade into insignificance when compared with the resting phase in rotifers and tardigrades which have been recorded by Rahm (1922) in Germany to be able to withstand a temperature of  $2^{\circ}$  absolute ( $-271^{\circ}$ ). The researches of Robinson and Miss Payne in America, and of Sacharov in Russia, have shown that cold resistance in insects is explainable on certain biophysical principles. Robinson's experiments showed that cold hardiness cannot be acquired by an insect rapidly—it requires a falling temperature. As the temperature sinks below freezing-point the colloid particles in the tissues and body fluids withdraw and bind free water as films around themselves. This so-called bound or adsorbed water ceases to behave as ordinary free water since it does not freeze even at  $-20^{\circ}$ . It is the ratio of bound to free water, which, when sufficiently high, protects insects against freezing temperatures. In pupae of the moth (*Telega polyphemus*) Robinson found that the bound water increased 48 to 50 per cent. when the insects had become fully cold hardened. Miss Payne (1929) has stressed the importance of humidity, and her numerous experiments indicate that cold hardiness bears an inverse relation to absolute humidity, regardless of temperature. While Robinson's obser-

variations with regard to a falling temperature are correct, they are only a partial explanation. For a given temperature insects are acclimatised to, their survival at lower temperatures depends upon the amount of humidity present as is shown in the accompanying table (Table I). In the case of the beetle (*Synchroa punctata*), Miss Payne, by using thermoelectric methods, found that when the insects were placed in a desiccator at 15° their freezing and undercooling points were greatly lowered. Thus, in the normal larvae, these points were  $-3.04$  and  $-6.71^{\circ}$  respectively, whereas in the desiccated larvae these same points were  $-71.4$  and  $23.26^{\circ}$ . It therefore appears that the moisture content of the insect itself is also an important factor in the process. Now the water content of insects is known to have a direct relation to that of food (Table II). Grain weevils have a water content of 45–50 per cent.; in the cabbage butterfly larvae it is 83–84 per cent. Miss Payne also found that cold resistance is greater in starved larvae and lower in those fed with moist food. Her conclusions have been confirmed, and in some directions amplified, by the work of Sacharov (1930). This experimenter paid particular care to the technique employed. He avoided the insertion of thermopile needles into the insects by employing the dilatometer and cryohydrate solutions. In this way surgical shock, which causes a measurable rise in temperature, was avoided. Sacharov's conclusions are apparently based upon careful technique and they may be briefly summarised as follows. Cold hardiness depends upon two intrinsic factors: (1) the proportion of easily freezable water to the total water content, and (2) the percentage of fat present. Hibernating insects, he claims, fortify themselves by reducing the one and increasing the other. He tested these two factors in the caterpillars of the brown-tail moth taken straight from hibernation and after feeding for several days in a warm chamber. The differences are very striking, and his results are quoted below (Table III).

Sacharov further carried out a number of observations upon different insects, taken from their natural habitats during winter, and subjecting them to different temperatures. His results are shown in abstract in the accompanying table (Table IV). With chafer larvae he points out that the reason for their penetration to greater depths in the soil during hibernation appears to be due to their possessing but a limited capacity for cold hardening. They would seem to have an exceptionally high water content and even at a temperature no lower than  $-5.7^{\circ}$  the amount of non-frozen water was found to be only 25.7 per cent. of the total water. The hive bee is well known to be susceptible to the effects of cold and damp and requires well-insulated quarters for hibernation.

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In this connection Sacharov's results are rather unexpected, since it would seem that this insect is more resistant than chafer larvae. The adult of the herald moth (*Scoliopteryx libatrix*) and the larvae of the longicorn (*Plagionotus arcuatus*), are both highly resistant to the cold. In the case of the herald moth, which hibernates under bark, in thatch buildings, etc., Sacharov mentions that it has been found coated with ice and yet subsequently revived and became active. It will be observed that its total water content is very low and 71 per cent. of this water was non-frozen at  $-11.1^{\circ}$ , while the ratio of fat to live weight appears to be high.

Table I.

*Water content of various insects and their food (from Robinson, 1928).*

Species	Food plant	Water content of food %	Water content of insects %
Granary weevil, adult ( <i>Sitophilus granarius</i> )	Stored wheat	9-11	46-47
Rice weevil, adult ( <i>Sitophilus oryza</i> )	Stored wheat	15-16	48-50
Locust borer, adult ( <i>Cyrtene robiniae</i> )	Locust tree trunk	30-32	56-60
Colorado potato beetle, adult ( <i>Leptinotarsa decemlineata</i> )	Potato plant, leaves and stem	70-74	62-66
White grub, larva ( <i>Phyllophaga</i> spp.)	Wheat shoots and roots	64-67	73-82
Mourning cloak, larva ( <i>Vanessa antiopa</i> )	Willow leaves	70-73	77-79
Willow sawfly, larva ( <i>Cimbex americana</i> )	Willow leaves	70-73	79-82
Cutworm, larva ( <i>Chorizagrotis</i> )	Lettuce	78-79	83-88
Army worm, larva ( <i>Cirphis unipuncta</i> )	Corn leaves and stem	77-78	87-89
Imported cabbage worm ( <i>Pontia rapae</i> )	Cabbage	88-89	83-84
Polymephus moth, larva ( <i>Telea polymepus</i> )	Hazel leaves	71-73	90-92

Table II.

*Cold hardiness in tussock moth eggs (from Payne, 1929).*

Environmental temperature $^{\circ}$ C.	Survival temperature	Relative humidity in %	Absolute humidity
20	- $1.5 \pm 0.5$	100	17.5
	- $6.1 \pm 1.3$	80	13.9
	- $11.5 \pm 2.4$	50	9.0
15	- $7.0 \pm 1.7$	100	12.8
	- $10.0 \pm 2.25$	80	10.2
	- $13.5 \pm 3.1$	50	6.5
10	- $11.0 \pm 2.3$	100	9.2
	- $13.3 \pm 3.2$	80	7.4
	- $16.5 \pm 5.6$	50	4.6
0	- $16.5 \pm 4.1$	100	4.6
	- $17.0 \pm 3.0$	80	3.5
	- $20.0 \pm 5.3$	50	2.2

Table III.

*Freezable water and fat in caterpillars of the brown-tail moth,  
Euproctis chrysorrhoea L. (from Sacharov, 1930).*

Temp. ° C.	Water %	Dry substance %	Ratio of frozen water to live weight	Ratio of frozen water to total water	Non- frozen water	Fat Ratio to live weight	Ratio to dry weight
A. Caterpillars from hibernating nests.							
- 5.75	71.83	28.17	—	—	100	4.93	16.44
- 7.8	"	"	—	—	100	"	"
- 11.1	"	"	3.63	5.06	94.94	"	"
- 17.35	"	"	10.93	15.22	84.78	"	"
B. Caterpillars fed for 3-4 days.							
- 3.9	82.94	17.06	—	—	100	2.52	12.46
- 5.75	"	"	4.13	4.98	95.02	"	"
- 7.8	"	"	37.20	44.85	55.15	"	"

*Note.* In series A the caterpillars withstood a temperature of -17.35°, while in series B 82 per cent. succumbed at -7.8°.

Table IV.

*Freezable water and fat in various insects (from Sacharov, 1930).*

Temp. ° C.	Water %	Dry substance %	Ratio of frozen water to live weight	Ratio of frozen water to total water	Non- frozen water	Fat	
						Ratio to live weight	Ratio to dry weight
Larvae of <i>Melolontha hippocastani</i> L.							
- 5.75	79.16	20.84	59.13	74.27	25.73	6.07	29.80
Adults of <i>Scoliopteryx libatrix</i> L.							
- 5.75	48.65	51.35	—	—	100	18.18	56.31
- 11.1	"	"	13.97	28.91	71.19	"	"
- 17.35	"	"	25.94	53.39	46.61	"	"
Larvae of <i>Plagionotus arcuatus</i> L.							
11.1	54.12	45.88	—	—	100	14.36	30.05
17.35	"	"	1.80	3.53	96.47	—	—
Adults of honey bee, <i>Apis mellifera</i> L.							
- 2.9	74.05	25.95	2.58	3.48	96.52	2.66	10.26
- 5.75	"	"	27.36	26.97	36.03	"	"
- 11.1	"	"	54.72	73.92	26.08	"	"

The work of the three observers mentioned has put us on the track of understanding the way of solving the problem of cold hardiness in insects and that it is linked up with the biophysics of metabolic water in the living creatures. All that we know about this water is that in cold hardy species a high proportion of it is held in a peculiar phase that does not freeze until relatively very low temperatures are attained.

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Whether this water is actually bound or adsorbed in the strict physical sense will not be discussed here.

In a state of nature we may picture to ourselves what appears to happen to an insect when it enters into hibernation. It ceases to feed, and wanders until it discovers an appropriate resting place. At this period it has a liberal store of fat in its tissues that has accumulated during the preceding period of activity. Its water content is reduced and, as the prevailing temperature of its environment gradually falls away during autumn and early winter, the ratio of non-freezable to total water in its body increases in relation to the fall in its freezing and supercooling points. Cold dry weather, it would seem, aids the acquisition of the insect's powers of resistance, whereas cold damp weather is much less favourable. It is probable that vast numbers of insects perish when the acquisition of cold hardening is interfered with, since the fatal supercooling point is higher and more quickly reached. The farmer, in cultivating his land during winter, disturbs the winter quarters of immense numbers of insects. These are often brought in the process into contact with much lower temperatures than their hardening capacity enables them to resist, and large numbers are killed.

*Variable temperatures.* Passing now to the investigation of variable temperatures, a field inquiry is entered upon which also has a bearing upon applied problems. The experimental study of varying temperatures has not, however, progressed very far in connection with insects. Obviously, much depends upon the length of exposure to given temperatures and the ranges of the latter. For particular insects there is a certain amount of experimental evidence that fluctuations between heat and cold influence rate of development by increasing it. This indicates that the idea of temperature summation based upon mean daily temperatures in phenological work may be beset with serious errors. Thus, both Bodine (1925) and Parker (1929-30) found that if the eggs of grasshoppers were transferred to  $0^{\circ}$  immediately after they had been laid and were later removed to a favourable temperature ( $27-37^{\circ}$ ) they developed more rapidly than the controls which remained at the higher temperature. This acceleration was progressively maintained up to a period of 240 days at the low temperature. Longer exposure resulted in only slight further acceleration. Kept at  $37^{\circ}$ , the eggs hatched in 46 days. When placed at  $0^{\circ}$  for 30 days and subsequently transferred to  $37^{\circ}$  they hatched in 37 days. There was progressive acceleration up to 240 days at  $0^{\circ}$ , after which they hatched in 11 days at  $37^{\circ}$  (Table V). It might be argued that  $0^{\circ}$  is above the threshold of development and

that growth was going on all the time, but as a matter of fact the threshold is above  $8^{\circ}$ , so that the result obtained is only to be explained as acceleration.

Table V.

*Development of grasshopper eggs at higher temperatures following an exposure to  $0^{\circ}$  C. (from Parker).*

Days at $^{\circ}$ C.	Days taken for hatching at temperatures given		
	$27^{\circ}$	$32^{\circ}$	$37^{\circ}$
0	26	32	46
30	25	31	37
60	23	29	31
120	20	21	17
180	17	18	15
240	16	12	11
500	15	10	10

Cook (1927) carried out extensive experiments with the first instar larva of the cut worm (*Porosagrotis orthogonia*), adopting a low temperature of  $8^{\circ}$  and subjecting the larvae for various durations at a series of high temperatures ranging from  $22$  to  $37^{\circ}$ . In every case he obtained a marked acceleration of growth during that instar as a result. His conclusions, however, are open to some criticism, since a certain amount of growth undoubtedly took place under the low temperature which was very close to the threshold of development. The growth, however, in most cases, was so much accelerated that a considerable balance would be left over after due allowance for this experimental error.

The question now arises as to what effect is produced by variations in temperatures that lie along the straight line portion of the velocity curve—i.e. those above the threshold of development. Headlee's experiments in U.S.A. with the grain aphid (*Toxoptera graminum*) and the codling moth (*Cydia pomonella*) are well known. It will be recollected that development at daily fluctuations between  $10$  and  $24.4^{\circ}$  reduced the rate of growth in the case of the codling moth by 8 days as compared with controls that were maintained at  $17.2^{\circ}$ , which is the mean of those two temperatures. Very similar results were obtained by him for the grain aphid. Parker obtained different results with the eggs of grasshoppers, positive evidence being shown that alternation of two medial temperatures accelerates development, as compared with subjection to the higher of the two temperatures employed. The greatest acceleration was produced, however, where the lower temperature was definitely below the threshold point. The most recent worker in this field



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is Ludwig (1928) who employed the different developmental stages of the Japanese beetle as material for his experiments. The general conclusions derived from separate studies of the eggs, larvae of different instars, pupae, and adults were that if the alternation were between one temperature below the threshold, and the other between that and the optimum, development is accelerated. No appreciable difference was observed when the temperatures lay between the threshold and optimum points, while, when the higher temperature lay above the optimum, retardation took place. The results so far referred to deal with species that hibernate in one or other phase of this life cycle. There remains to be considered the reaction to alternating temperatures of species that undergo no actual hibernation, and are subjected, in a state of nature, to relatively small temperature fluctuations. Information on this aspect of the subject is scanty and fragmentary. In the case of the Mediterranean flour moth (*Ephesia kuhniella*) and the bed bug (*Cimex lectularius*) exposure of the eggs for a period at low temperature definitely delays their hatching at the higher temperature. There are other instances where, apart from any inhibiting effect upon growth, malformations have resulted. It is possible, in these cases, the temperatures employed were near the zero point for the basal metabolism for the species concerned, but there is too little exact information to allow of generalisation.

In well-accredited experiments with alternating temperatures, in which an accelerative influence upon growth is known to take place, it appears to be questionable whether such acceleration is not more apparent than real. It is possible that growth under such circumstances more nearly approaches natural conditions than under the influence of a given constant temperature. Variable temperatures are the rule in nature and it is, therefore, an open question whether or not subjection to a constant temperature results in an actual retardation of development. Most insects in nature are, as it were, attuned to seasonable temperature rhythms, and it would appear that, in certain cases, at least, subjection to a period of low temperature is essential to normal development. Thus, the eggs of certain grasshoppers require a diapause at low temperatures to initiate development. It is also well known that commercial rearers of silkworms subject the eggs to a temperature just above freezing-point for several months in order to ensure a regular hatching of the larvae.

The practical implications of such work on alternating temperatures is of concern to phenological observations entailing temperature summa-

tion above a given threshold. The temperatures involved in these computations are mean daily temperatures: so long as the extremes of these temperatures lie along the straight line part of the velocity curve all may be well and good. But when the mean is drawn from a range outside these limits, each day degree may acquire a totally different value, and erroneous conclusions be drawn.

#### HUMIDITY.

*The general subject.* It has already been shown that recent research has established two definite features with respect to the influence of humidity upon insect life. Firstly, that it plays an important part in connection with cold hardiness in different species. Secondly, that the water balance in the bodies of insects is dependent to a considerable extent upon the water content of their food. We have to recollect that the surface areas of these creatures is relatively great as compared with their volume and consequently the problem of water conservation must be a significant one, particularly in species living in abnormally dry situations. The rate of water loss from the tissues appears to be governed to a certain extent by the humidity of the atmosphere, but critically controlled experiments upon a sufficiently large scale to neutralise experimental errors are few. Different insects, we know, can suffer variable amounts of desiccation through evaporation, without loss of viability. Certain chafer larvae are known to be unable to survive a loss of 15 per cent. of their body weight of water, while others can sustain a loss up to 80 per cent. Buxton (1930) has recently shown that mealworms after 1 month without food are able to maintain a nearly constant proportion of water in their bodies at humidities ranging from 0 to 60 per cent. This fact is one of great importance as regards survival under conditions deviating from the normal and, according to the theory of Robinson, there is a definite amount of what might be called stabilised water varying in different species of insects. This water, he believes, is bound or adsorbed around the colloid particles of the tissues. Thus in grain weevils, for example, the total water content is only 46–48 per cent. of their weight, but 35–50 per cent. of it is in the bound phase. From Buxton's work, already alluded to, it would appear likely that it is the water in this phase that remains tolerably constant and enables grain insects to withstand dry conditions. On the other hand, a species such as *Sitophilus granarius* is unable to withstand cold, since the ratio between the bound and free water alters little with falling temperatures.

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At 0° Robinson found that a rapid drop in the so-called bound water occurred.

Among recent researches dealing with the influence of humidity upon insect life particular mention may be made of the work of Parker (1929, 1930) on grasshopper eggs. He showed that the highest percentage of nymphs emerged from eggs at 22° and 80 per cent. relative humidity. Higher temperatures required a higher humidity, but the range of tolerance to humidity alterations was markedly restricted. As regards rate of development, at a constant temperature of 27°, development was quicker at humidities ranging between 60 and 100 per cent. In the case of eggs kept at 23–25° the reverse obtained, eggs at the lower humidities developed in the shorter times. This marked difference is explained by Parker on the supposition that high temperatures and low humidities reduced the water content of the eggs more rapidly, and that this reduction was responsible for retardation in development.

*Saturation deficiency.* At this point it is necessary to refer to the work of Bacot and Martin (1924), whose carefully planned experiments showed that, in the case of the Indian plague flea (*Xenopsylla cheopsis*), duration of life was directly proportional to saturation deficiency, when a number of experiments are carried out at a given constant temperature and over a range of different saturation deficiencies. When, however, very high temperatures were reached this no longer held good: other factors came in and, if saturation deficiency alone held good, in fully saturated air the flea would be immortal!

In a communication read before the Empire Conference of Meteorologists by Prof. V. H. Blackman, stress was laid upon the desirability of taking saturation deficiencies into account rather than relative humidities in phenological observations. Relative humidities give no indication of the evaporating power of the air. Air at 70 per cent. relative humidity and 20 and 30° temperature has saturation deficits of 5.22 mm. and 9.47 mm. of mercury respectively, and its evaporating powers are consequently very different. We have at least some evidence that water loss in insects, as in plants, depends to a considerable extent upon this factor. Buxton has recently emphasised this subject from the entomological standpoint in a useful paper published in 1931 wherein various methods of technique for the measurement and control of humidity are discussed. In a second article he has analysed the work of Parker and several other observers in terms of saturation deficiency. In the accompanying graph (Fig. 2) it will be observed that, in regard to the hatching of the eggs of the grasshoppers (*Melanoplus*), air that is

just dry enough to kill 80 per cent. of the eggs is half saturated at 22° and four-fifths saturated at 37°. Across Parker's table lines representing saturation deficiencies of 5 mm. and 10 mm. respectively have been drawn. There is, it will be noted, a tolerably good fit between the lines and Parker's data. In other words, the data show that the proportion of eggs which produce nymphs bears a relation to the saturation deficiency of the air within the temperature limits explored by Parker.

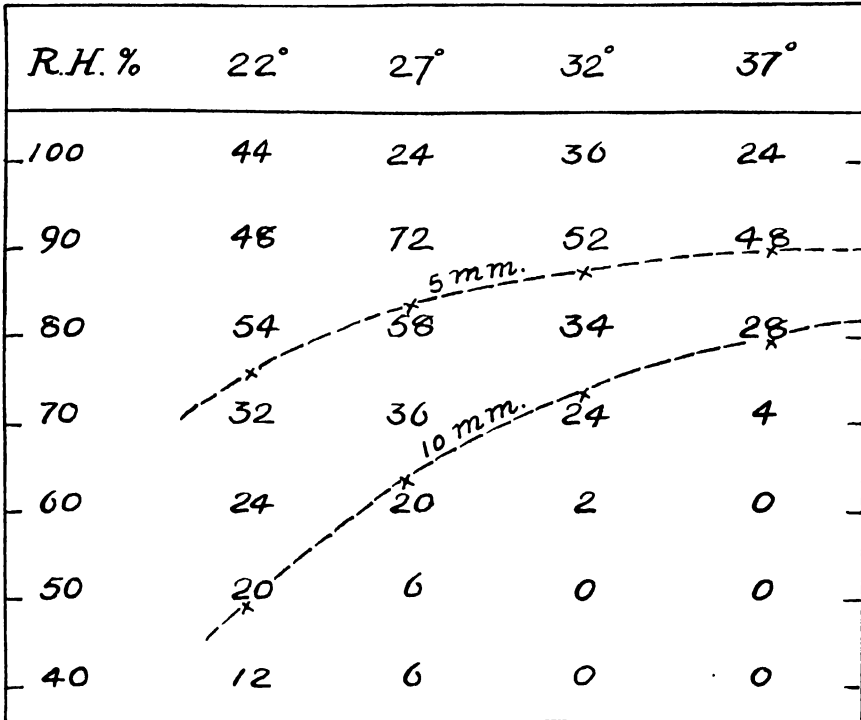


Fig. 2. Table showing percentage of *Melanoplus* eggs hatching at different constant temperatures and constant relative humidities: based on data by Parker (1930). The lines of 5 mm. and 10 mm. saturation deficiency have been drawn across the table. The figure is adapted from Buxton (1931).

The subject of saturation deficiency is discussed with the object of emphasising the necessity for more attention being paid to it. With scarcely any exceptions, all the enormous literature dealing with temperature and humidity in relation to insects is concerned with relative humidities. Quite a considerable amount of work has been done over a series of temperatures but at the same relative humidity. Now it

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would seem that, had a uniform saturation deficiency been adopted for each of the temperatures, results would have been obtained that would have given the measure of the effects of different temperatures in air of the same evaporating powers. However, whether this conclusion be correct or not, when sufficient controlled experiments are conducted on a scale adequate to eliminate experimental errors due to individual variations betrayed by the insects themselves, the physiologist will come into possession of data that will enable him to discover which method of measurement yields the best comparable results.

### CONCLUDING OBSERVATIONS.

The last fifteen years or so have witnessed the publication of a large number of papers containing observations relative to the effects of temperature in diverse ways upon insect life. Such information has come, in the main, as the outcome of practical investigations emanating from state or state-aided institutions, where provision of the necessary apparatus, and the maintenance and rearing of large numbers of insects, offer fewer difficulties than elsewhere. Applied entomologists are no longer content with the concentration of their efforts upon life histories and direct control measures. They are turning more and more to the study of living animals in relation to their environment. An ecological conception is thus coming into being which recognises an injurious insect as being the product of a particular environment. This is leading more and more to attempts to analyse the effects of individual environmental factors. It is becoming to be recognised that, if we can in any way foresee the contributing causes of insect outbreaks, we are likely to be in a better position to contend with the latter. It is the old adage—to be forewarned is to be forearmed. Of the many environmental factors at work climate and weather stand out in special prominence because they are ever present and not amenable to control.

In so far as weather is concerned, a definite study which we may call insect epidemiology has arisen in recent years. It consists in attempts to correlate particular weather conditions with the prevalence of individual pests. Examples of such correlations are well known to all entomologists but, although circumstantial evidence is strong enough in some cases to amount almost to a certainty, they have little backing in the way of fundamental knowledge. We can seldom evaluate the direct effects of weather upon insect prevalence, as distinct from its indirect influence operating through such potent biological factors as food plants, parasites, predators and disease organisms of various kinds. In a few words, insect

epidemiology aims at prediction being made with a degree of certainty that would warrant the preparation in advance of appropriate measures. The collection of data of this kind is possibly best organised and most advanced in Germany. In the recent publication of Schnauer (1929) a very large amount of information, systematically collected, year by year, through the auspices of the German Agricultural Society since 1893, is summarised and analysed. The causes of the outbreaks of major pests are surveyed in conjunction with data respecting the chief meteorological factors. The information thus accumulated has enabled it to be possible to trace the probable connection of fluctuations in the prevalence of specific pests with particular weather conditions. This data is now being utilised as the basis for future forecasting.

Investigations of temperature and humidity bear a distinct relation, not only to the forecasting of pest outbreaks, but also to the forecasting of the probable distribution of species. It needs no elaborate data to show that the geographical range of, at any rate, certain insect species is no longer restricted by purely geographical barriers. The advent of rapid communication by land and sea and air has opened up new problems which reflect themselves in the rigid quarantine laws that so many countries have had to enforce. It has so come about that it is no longer sufficient to take cognisance of the existing distribution of insects known to be pests. The zones of their possible future distribution cannot be wholly neglected, at least by the applied entomologist. Study of climatic conditions prevailing over the area covered by the existing range of a species will enable certain general conclusions to be drawn with regard to its limits of climatic tolerance. The influence of the individual factors upon that species can, to some extent, be subjected to experimental laboratory analysis. In this connection the recent appearance of Dr Royal Chapman's new book on *Animal Ecology* will be welcomed as a useful practical guide. The results of this combined method of approach, namely, field and laboratory, are well reflected in the case of the Mediterranean fruit fly (*Ceratitis capitata*). If all the areas known to be permanently infested by this insect be plotted on a world map, they are seen to be almost entirely confined to the tropical and sub-tropical belt bounded by the mean January isotherm of 10° north and south. Outside these limits it only occurs constantly on the northern Mediterranean coast. Possibly the isotherm line which, after all, is much smoothed out, actually embraces this particular area. Anyway, laboratory studies by Back and Pemberton, and more recently by Bodenheimer, have given us a tolerably clear idea as to the range of temperatures tolerated by

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this insect. Its threshold of development is approximately  $13.5^{\circ}$ , consequently the development will be arrested once the temperature falls appreciably below this point. Before the threshold had been determined, it had been previously concluded that there was no evidence that the Mediterranean fruit fly would prove a pest in any country where the mean monthly temperature falls to or below  $10^{\circ}$ , for three or four consecutive months. Complete mortality follows such exposure. A fortnight's voyage in a refrigerating chamber kept at freezing-point will kill the eggs or larvae of the insect. For temperatures along the straight line portion of the velocity curve Bodenheimer found that the thermal constant is 250. We have sufficient data to show that the insect will attain its maximum proportions as a pest in regions where the average temperature does not fall below  $13.5^{\circ}$ . This condition is fulfilled in most tropical regions where the insect passes through uninterrupted generations, one following the other, all through the year. In England it cannot become acclimatised because the number of days in the year with effective temperatures is not sufficient for even a single generation to develop. In the Mediterranean region the insect has become established, but its severity as a pest does not equal its behaviour in the tropics. The main factor seems to be the winter, wherein the temperatures, though not lethal, exercise severe restraint upon the species by reducing the number of generations possible within a year. At the mean winter temperatures of this region a simple generation occupies  $3\frac{1}{2}$  months, whereas in the tropics a number of generations would be passed through in that period. We know from data of this kind that there are many countries wherein the insect might readily become established. Florida, the West Indies and Fiji, to mention just a few, appear to provide the requisite environmental conditions. A few years ago the insect appeared in Florida for the first time—from an undetermined source—and great alarm was caused throughout this rich citrus-fruit area. Happily, the expenditure of a vast sum of money, and the application of most energetic repressive measures, are stated to have led to a successful outcome before the species had obtained an established foothold.

In the foregoing remarks it has only been possible to refer to certain aspects of the influence of temperature and humidity upon insect life. If it has been made evident how recent research has served to emphasise the importance of the experimental study of these two factors, in relation to applied entomology, it is hoped that this address will have served the purpose intended.

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(Received February 26th, 1932.)



## STUDIES IN THE PHYSIOLOGY OF VIRUS DISEASES IN PLANTS

### III. AUCUBA OR YELLOW MOSAIC OF TOMATO IN *NICOTIANA GLUTINOSA* AND OTHER HOSTS

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(With Plate V.)

THE purpose of the present paper is to put on record some experiments with aucuba or yellow mosaic of tomato which have been carried out on solanaceous hosts and to direct attention especially to the use of *Nicotiana glutinosa* with this virus, in experiments of a more general nature.

A detailed description of the symptoms induced by aucuba mosaic in tomato (*Lycopersicum esculentum* var. Kondine Red) has already been given (1). In tomato, *Nicotiana rustica*, *Solanum nodiflorum*, and *S. nigrum*, this virus induces mottling in the leaves and, occasionally, in the fruit. Similar symptoms appear in tobacco (*N. tabacum* var. "White Burley") but this host is exceptional in that it also develops local symptoms at the point of inoculation. In all these plants intracellular inclusions have been found. Further, the disease is systemic and the symptoms are not confined to the region of inoculation.

The chlorosis which is the typical symptom in these host plants is apparently due to an inhibition of the formation of chlorophyll, rather than to an actual destruction of the preformed chloroplasts. This is clearly indicated by the observation that chlorosis affects the leaves round the growing point, *i.e.* those leaves which have developed *after* the inoculation of the plant.

Inoculation is effected by rubbing the leaves of the experimental plants with macerated tissue of infected tomato leaves. No local lesions appear as a consequence of this treatment except in tobacco, which is remarkable in its reaction to this disease. In the plants under discussion, there is little alteration in the starch content of the cells of leaves which were mature at the time of infection (*cf.* Holmes (2)). When, however, leaves which had developed or which were developing symptoms of mosaic are treated with alcohol to remove the chlorophyll and then

with iodine, the absence of starch from considerable areas can readily be demonstrated. There is apparently little starch in the regions which are chlorotic, and very often the vascular bundles are included in those regions. In older leaves, which do not show symptoms, the starch is uniformly distributed over the tissues.

In another group of solanaceous plants, however, the symptoms induced by inoculation with aucuba mosaic juice are quite different since the primary symptoms appear on the rubbed leaf. The rubbing of the leaf breaks the hairs and through the broken hairs the virus agent enters the tissues. Within five days in the Rothamsted Experimental Station glasshouses necrotic spots appear on the treated leaves. They are generally distributed over the leaf-surface and are associated with the broken hairs of the adaxial side. These spots are about 5 mm. in diameter, are roughly circular in outline and increase only slightly in size for a few days. The tissues are shrunken and dry and the leaves are consequently distorted and crinkled by the collapse of the cells. No other symptoms are visible on the treated plants and no systemic infection occurs. Reactions of this type are found in *Datura stramonium*, *N. acuminata*, and *N. glutinosa* of the species which have been examined.

*N. tabacum* occupies an intermediate position between the first and second groups, in that local lesions appear on the rubbed leaves and, later, mosaic symptoms appear systemically over the whole plant. In the second group no intracellular inclusions are formed in the cells of the affected leaves and the virus is not generally recoverable from tissues of the host plant other than those of the leaves inoculated in the first instance. No movement of the virus takes place across a leaf of *N. glutinosa* of which only a portion has been rubbed with virus juice.

#### THE MOVEMENT OF THE AGENT ACROSS THE LAMINA.

A series of leaves of *N. glutinosa* were rubbed, some on the distal portion of the lamina, others on the proximal portion and others on one side of the midrib. In no instance was any virus recovered from the unrubbed portion of the leaves. When minimal doses were rubbed on leaves and, after symptoms had developed, these leaves were macerated with water and inoculations were made back into tomato the majority of the plants did not become infected. Since tomato is so easily infected, this suggests that the amount of virus present in the *Nicotiana* was small.

Plate V, fig. 1 shows the appearance of a plant of *N. glutinosa* inoculated by rubbing virus on the four lower leaves. The photograph was taken a fortnight after treatment and it can be seen that the rubbed

leaves developed well-marked symptoms of the disease and that the rest of the plant was normal.

It was noticed that in our glasshouse occasional old plants of *N. glutinosa* developed mottled leaves at the top. This mottle occurred on some of the plants with rubbed leaves, and systemic secondary infection was suspected. Inoculations were made from this material into tomato and on the leaves of other plants of *N. glutinosa*. No symptoms developed on these plants so that, despite the superficial resemblance, the mottle was not causally associated with the aucuba mosaic.

That this species may develop systemic infection with other viruses has been clearly shown. When inoculations were made by rubbing inocula of "experimental streak" on the lower leaves local lesions appeared within a few days and systemic infection occurred later. The systemic infection was a fine mottle induced by the potato mosaic virus which was a component of the "experimental streak," the latter being a mixture of the virus of aucuba mosaic and of potato mosaic. The mixed viruses induce systemic mosaic and necrotic symptoms in the tomato. The virus of aucuba mosaic in *N. glutinosa* therefore induces similar symptoms to those found by Holmes for the virus of tobacco mosaic. Holmes has suggested that the number of spots on the rubbed leaves of *N. glutinosa* and *N. rustica* is an index of the concentration of the virus agent in the infective material(3).

The "drying out" of the areas on the leaves of *N. glutinosa* resembles closely the "drying out" which occurs not infrequently on the leaves of tomato plants infected with severe aucuba mosaic. The characteristic feature of this "spotting" is that the tissues are brown rather than black, since little melanin pigment is formed in the dead areas as occurs, for instance, in "streak" disease. The tissues are desiccated and their appearance suggests a severe local wilt. Similar symptoms may appear in tomato plants infected with aucuba mosaic when kept in the glasshouse, and do appear if similarly infected tomato plants are kept in the dark for some days.

The fact that leaves of *N. glutinosa* react to aucuba mosaic as they do to tobacco mosaic indicated that they might well be used for the demonstration of the presence of this virus in a juice. It had previously been noted that the virus of aucuba mosaic multiplies and induces symptoms in young detached tomato leaves kept with their petioles in damp sand. It seemed reasonable to suppose, therefore, that if *N. glutinosa* leaves were detached from the plant, inoculated by rubbing and left in Petri dishes or on damp sand, symptoms would develop after the usual period of inoculation.

For the purpose of the experiment infected tomato leaf tissue was macerated with distilled water in the proportion of 1 gm. of leaf tissue to 2 c.c. of water. This material was passed through a fluted filter paper impregnated with fuller's earth. The filtrate was dark brown in colour and contained no chloroplasts. Inoculum prepared in this way was considered as the standard inoculum in all the experiments recorded in this paper and all dilutions were made on that basis.

#### THE DETECTION OF THE PRESENCE OF THE VIRUS AGENT IN JUICE.

Filtered virus juice was diluted to 1/10 and two drops of this material were rubbed on the surface of detached leaves of *N. glutinosa*, which were kept with their petioles in damp sand under a bell-jar in the glass-house. Within 48 hours the first sign of "spotting" appeared on the leaves. Control leaves which had been rubbed with water or with healthy juice were normal. The rubbing was done with the index finger, care being taken to rub the inoculum evenly over the surface without damaging the rather soft tissues of the mesophyll. On the third day, that is within 72 hours, the rubbed areas were dry and brown. The appearance of the leaves is shown in Plate V, fig. 2. Excellent results have been obtained by rubbing the surface of the leaves with juice diluted to 1/100 and thereafter laying the leaves on damp filter paper on the bottom of Petri dishes. These were left on the laboratory table and definite symptoms appeared within 48 hours. This technique is valuable in that it affords a quick and reliable method of determining the presence of the agent, at least of aucuba mosaic, in a suspected juice. This method has the further advantage of approximating even more closely to the methods of culturing bacteria on plants than does that of Holmes(3), from which it is adapted.

Little importance can be attached to the conditions under which the detached leaves are maintained, but they must always be kept moist either in the light or in the dark. There is some evidence, however, that if they are kept under too wet conditions the appearance of symptoms may be delayed or partially suppressed. This suggests that wilting is the main factor in the development of symptoms. The number of spots varies considerably with various factors (cf. Holmes) but the number is always large enough to demonstrate the presence of the virus. Mere mechanical injury does not cause the "spotting." The area of the groups of dead cells does not increase in size after the first few days but remains more or less unchanged after they have become delimited. The cells of the leaves do not contain the intracellular inclusion bodies often found in

association with the disease, nor do the other cells of the plant present any abnormal appearance. There is no evidence of much, if any, multiplication of the virus in the affected leaves.

This latter point is difficult of exact assessment in that there is always a certain amount of virus juice adherent to the rubbed surface. On the other hand, when the inoculum was used in low dilution, *e.g.* 1/10, in no experiment did more than a few of the experimental plants secondarily inoculated develop symptoms. After rubbing, the leaves of the *Nicotiana glutinosa* were washed in a stream of water to remove all the surplus inoculum which adhered to the surface. The material was inoculated into groups of eight tomato plants and, at most, two or three in each group developed symptoms, while sometimes no plants of the group showed any trace of symptoms. When the *N. glutinosa* leaves were treated with alcohol to remove the chlorophyll and were put into an aqueous solution of iodine in potassium iodide, starch was found to be present in the cells of the lamina except in the region immediately round the lesions. There was a small "halo" of tissue without stain round each lesion but no spread of the clear tissue down the neighbouring vascular tissue as found in tobacco leaves by Holmes.

The appearance of the spots can readily be seen in the photograph on Plate V, fig. 2. On the leaf photographed the area of dead tissue is clearly delimited and the damage is purely local.

Two hypotheses suggest themselves in the interpretation of these phenomena. Either (*a*) the effect of this virus, which in so many of the Solanaceae causes definite mottling, is completely changed in this particular plant by some substance or condition which is not found in the others, or (*b*) the immediate effect on the tissues of this plant is so violent that a region of tissue round the point of inoculation is completely killed so that no virus is able to enter living cells of the mesophyll (*cf.* Caldwell(4)).

#### THE MOVEMENT OF THE VIRUS AGENT INTO UNBROKEN CELLS.

Various experiments have indicated that the virus agent is unable to enter unbroken cells. It has been found that infection does not follow wetting the surface of the leaf of tomatoes etc. with aucuba mosaic juice, nor does it occur when juice is forced directly into the xylem vessels(4). It appears, therefore, that the epidermis of the leaf and the walls of the vessels and of the living cells round them act as barriers to the agent. Similarly, the root-hair seems to be impervious to the agent while the wall is intact, since it is possible to grow seedling tomato plants on cotton

wool soaked in virus juice and, though the roots are bathed in the juice, the plants grow fairly rapidly and remain free from infection. Again, watering plants with virus juice in no case has given rise to symptoms, though infection can take place through a broken root. If, however, inoculations be made from the roots of diseased plants there is every indication that the virus content of these roots is considerable.

The observations that infection does not follow wetting with virus the unbroken cells of the epidermis of a plant or of the vascular tissue or of the roots, support the view that the agent is unable to enter an unbroken cell of a plant. The objection could, however, be raised that the epidermis is covered with a cuticle which is largely water-proof, and that the junction between the xylem vessels and the living parenchyma is obviously not of a simple membrane type. The same objection does not appear to hold for the root-hair but, admittedly, the root-hairs are a rather specialised mechanism.

One type of cell seemed to be particularly suitable for experiments of this type and that is the mesophyll cell. Especially did the leaves of *N. glutinosa* appear to furnish useful material in that detached leaves of this plant show symptoms within 48 hours. It was assumed that these symptoms could and did appear only when rupture of the cells had taken place (cf. tomato experiments).

#### THE INJECTION OF THE LEAVES OF *N. GLUTINOSA* WITH VIRUS JUICE.

For these experiments the virus juice after filtration was diluted to 1/10 and was put into a glass vessel of suitable size. The leaves to be treated were removed from the plants so that no hairs on the laminae were broken, and the leaf was invariably held by the petiole. Through the end of the petiole at right angles to the lamina was pushed a long pin, by which the leaf could be suspended into the virus juice and the lamina completely submerged. The juice had no access to the damaged region of the petiole and the pin was so placed on the top of the jar that the lamina did not knock roughly against the sides. The vessel was put under a bell-jar attached to a suction pump and the air in this jar was then exhausted to a considerable extent whereby the gas in the intercellular spaces of the leaf was removed. When the tension was released the juice entered by the stomata and filled the intercellular spaces. The leaves under experiment weighed some 2-3 gm. and took up about 2 c.c. of juice, approximately their own weight of juice. The appearance of the leaf was naturally altered by this treatment, the leaf being darker in colour and almost translucent by transmitted light. The leaves were

set out to evaporate most of the superfluous juice from the intercellular spaces and were then fixed by the pins to frames under the bell jars which kept the air moisture-laden. Alternatively, the pins were removed and the leaves were laid on moist filter papers or cotton wool spread on the bottoms of Petri dishes. The removal of some, at least, of the juice in the intercellular spaces was especially necessary in the case of those leaves which were afterwards kept in Petri dishes, since the complete injection of the leaves apparently induced anaerobic respiration and this, in turn, led to the destruction of the tissues of the mesophyll. This consideration did not arise to the same extent in the less well-saturated atmosphere under the bell jars. In none of these leaves did any spots appear after the preliminary experiment. In this experiment five leaves were used and on each of three of these a single necrotic spot appeared. In the later experiments the leaves were invariably normal after treatment.

The controls were of two groups. Some leaves were treated in a manner similar to that described, with the exception that they were rubbed on the surface after treatment. "Spots" appeared on these leaves. Other leaves were rubbed with virus juice on the surface and, in these cases also, symptoms invariably appeared. The amount of virus rubbed over each leaf was approximately 0.1 c.c. and in these experiments at least 15 spots appeared on each leaf treated. The difference in the amounts of juice in the uninfected leaves and the infected rubbed control leaves was considerable. Nevertheless, in the leaves with the unbroken cells the larger quantity of juice was apparently unable to cause infection since there is no means of entry into the mesophyll cells.

#### THE MOVEMENT OF THE VIRUS IN PLANTS WHICH DO NOT DEVELOP SYSTEMIC SYMPTOMS.

Reference has been made to the fact that in some of the Solanaceae the agent of aucuba mosaic is apparently not found in quantity in regions other than those directly inoculated. An examination was made of the possibility of movement of the agent through tissues in which it was not producing symptoms. For this purpose an experiment was set up in which tomato shoots were grafted as scions on to stocks of *Datura stramonium* and *vice versa*. Four groups of plants were set up for the experiment, tomato plants, *Datura* plants, *Datura*/tomato grafts, and tomato/*Datura* grafts. Two leaves on each of the plants were rubbed with aucuba mosaic juice. In the first group of the grafts two leaves of the tomato portion were rubbed and in the second two leaves of the *Datura*.

In the ungrafted tomato plants no symptoms appeared on the treated leaves but systemic mosaic symptoms appeared, as usual, after ten days. In the ungrafted *Datura* plants necrotic lesions appeared in the rubbed leaves and, very occasionally, on the stem above a rubbed leaf. This latter phenomenon is not unusual, though no explanation suggests itself as to why the isolated necrotic areas should develop sporadically over the plant. Henderson Smith<sup>(5)</sup> has found that these are the regions from which the virus agent is recoverable on subsequent inoculation.

When the grafts were examined it was found that necrotic areas had developed on the rubbed leaves of the *Datura* after four or five days, and that mosaic symptoms subsequently appeared in the tomato whether scion or stock. When, however, the virus juice had been rubbed on the leaves of the tomato portion mosaic symptoms never appeared on the *Datura*.

In *Datura* therefore the presence of the agent passing through the plant is apparently not sufficient to induce the formation of the necrotic areas which are characteristic of the disease but that some other factor is involved in the expression of disease symptoms.

#### SUMMARY.

It has been shown that the symptoms induced by aucuba or yellow mosaic of tomato in certain other members of the Solanaceae (notably *N. glutinosa* and *D. stramonium*) differ markedly from those in tomato. Neither formation of intracellular inclusions nor systemic infection occurs in these plants. In *N. glutinosa*, the symptoms appear only on the rubbed portion of the leaves and little multiplication of the virus takes place. In *D. stramonium*, although no mosaic symptoms appear on the host, the virus travels through the tissues and can infect susceptible grafts. Holmes' work on the use of *N. glutinosa* as a ready means of demonstrating the presence of the virus agent in a juice has been confirmed and amplified.

It has also been shown that it is possible to inject the intercellular spaces of the leaf of *N. glutinosa* with virus juice and that no infection occurs unless cells have been ruptured.

This work was carried out under the auspices of the Empire Marketing Board.



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EXPLANATION OF PLATE V.

Fig. 1. Plant of *N. glutinosa* with symptoms of aucuba or yellow mosaic of tomato on the lower leaves.

Fig. 2. Leaf of *N. glutinosa* with symptoms of aucuba or yellow mosaic of tomato.

(Received October 10th, 1931.)



Fig 1

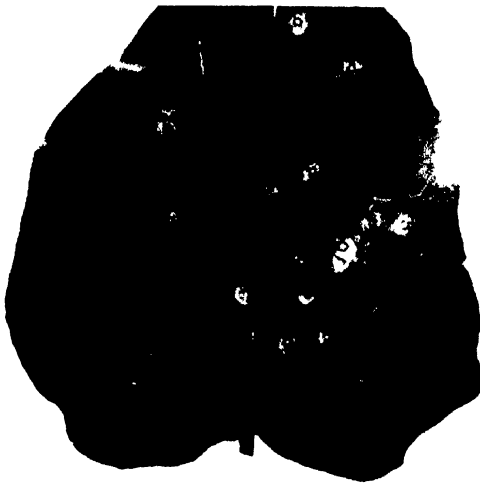


Fig 2



## “BREAKING” IN TULIPS. II

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(With Plates VI and VII.)

### INTRODUCTION.

THE first record based on experimental evidence that the contagious pathological condition known as “breaking” in tulips could be artificially induced by manipulation of the bulbs when in the so-called dormant condition was published in 1928(8). The results of the first flowering season after manipulation showed quite definitely that “breaking” was infectious and transmissible from bulb to bulb by bringing into contact and keeping in contact the freshly-cut tissue of a normal with freshly-cut tissue of a “broken” bulb. The results also established beyond a doubt that the mottling of the leaves and change from self-colour to variegation in the flower were due to the same cause.

Much about the same time Atanasoff(1) was able to show that “breaking” could be induced in the tulip by needle inoculation with freshly expressed unfiltered sap from the shoots of narcissus and hyacinth affected with mosaic. As far as is known, this is the only record of other hosts carrying the tulip “breaking” virus. Other publications on infectious variegation and insect vectors will be found in the References.

The “broken” tulip plant in general appearance presents all the symptoms of a virus infection; the leaves are mottled, the plant is less vigorous, the bulb does not proliferate so freely and the plant comes into full bloom a week or ten days later than the breeder; but, although pathological, “breaking” can hardly be looked upon as a definite disease. It is more a case of balance or tolerance of the host to the virus, as the plant does not succumb, but can live as a carrier for many years. For instance, the “broken” variety *Zomerschoon* was first described in 1620, and is still grown.

The virus in the narcissus and hyacinth, on the other hand, brings about a definitely diseased condition.

In the tulip, provided the plants were protected against aphid infestation, all injections into dormant bulbs of filtered sap expressed from

“broken” bulbs gave negative results. A tulip bulb of normal size can take in and retain as much as  $\frac{1}{2}$  c.c. of liquid if the injection is made into the chamber surrounding the growing point, and the bulb kept in an upright position after manipulation. The young growing point is thus bathed for a time in the infecting fluid and yet no effect is produced; whereas the close proximity of a small plug of infective tissue can bring about mild “breaking” the following flowering season. Thus it appears that, when outside the living cell, the virus is de-activated on exposure to the air for the period necessary for filtration, etc.; or it may be that it is adsorbed by the colloidal matter in the sap and cannot pass the filter.

In the previous paper<sup>(8)</sup> the statement made that “no tulip species has been known to ‘break’ either in the wild or under cultivation” must now be modified. It is true that there is still no record of the “breaking” of true species in the wild, but under garden conditions quite a number of different species have “broken” naturally in the collection of *Tulipa* species at the John Innes Horticultural Institution. Also “breaking” has been induced in two species, *Tulipa Greigii* and *T. Eichleri*, and in the somewhat doubtful species *T. gesneriana spathulata*, by bulb grafting with “broken” garden varieties. “Breaking” in *Tulipa* species is not very pronounced, and with the exception of *clear*<sup>1</sup> “breaks” in *T. linifolia* and *T. clusiana*, the “breaks” observed in all the other *Tulipa* species have been *self* “breaks.” Hence it is quite possible that “breaking” does occur in the wild, but has been overlooked.

Again, the mild form of parrotting recorded as having occurred in the plots of bulbs plugged with “broken” tissue in 1928 proved to be only a temporary disturbance and entirely disappeared in 1929. In the summer of 1930 (the third year after treatment), a season in which a noticeable amount of fasciation was observed in different species of plants, several cases of fasciation and ragged petals were recorded in these same plots; but here again these abnormalities disappeared almost entirely in 1931.

Recent results have shown that different varieties of transmitters can vary in degree of potency, and different species and varieties of host can differ in degree of susceptibility towards the same transmitter.

<sup>1</sup> The more comprehensive terms *self* and *clear* have been substituted for the terms used by McKenny Hughes, *red* and *white* respectively, to distinguish the two types of “breaking” found in tulips of all colours. The *self* “break” consists of irregular areas of concentrated pigment on the original self ground. The *clear* “break” consists of irregular areas of concentrated pigment and of self colour on a ground devoid of anthocyanin. The yellow or white ground colour depends upon the presence or absence of yellow plastids in the mesophyll cells.

## GRAFTING EXPERIMENTS OF 1927.

In the paper on "breaking" in tulips published in 1928, the results of the first flowering season after manipulation of the bulbs only were given. In this series of experiments three old-established "broken" varieties were used as transmitters, Kroeschel, Kaleidoscope, and a "broken" form of Sulphur. The host was the same throughout, a self-coloured crimson breeder, Bartigon.

All these three transmitters proved to be good, more especially Kaleidoscope, and the average percentage of "breaks" induced by these three transmitters was 26 per cent. in the first year.

The plants from these treated bulbs were harvested and recorded separately and replanted in the autumn of 1928. The 1929 records are given in Table I. It must be pointed out that no control measures were taken to keep down aphides in the first flowering season, and the plants were fairly heavily infested during the summer of 1928. The result was that two "breaks" appeared in the control plot in 1929. This control plot was situated in 1928 at the extreme end of the tulip ground, with another control plot of untreated bulbs on one side and a wide garden path on the other. Plot A, on the other hand, was between a control plot and a plot of treated bulbs showing 21 per cent. of "breaks"; plot B was between two plots showing 26.6 and 30.1 per cent. of "breaks" respectively; and plot C between a plot with 21 per cent. of "breaks" and another control plot showing none. All the plots were separated from one another by a 15-in. path.

This close juxtaposition of the treated plots to plots showing "breaks" no doubt accounts for a certain percentage of "breaks" in the second year, but even allowing for this, the evidence was fairly conclusive as to the infectious nature of "breaking" and its transmissibility by artificial means.

If the flower records are taken alone, the percentage of "breaks" during the two years is 79.7 per cent. as against 5.5 per cent. in the controls. But if leaf records are included then the percentage is considerably increased. There is no difficulty in detecting "breaking" in the leaves.

The "breaks" in A, B and C were used for grafting with parrot tulip bulbs in the autumn of 1929, but the controls were grown on for another year, after having been sprayed against aphids during the growing season of 1929. In the third year these controls gave a total for the three years of 8.3 per cent. of "breaks."



Owing to the damaging method of treatment, the number of flowering plants is very low, so that, if leaf and flower records are taken together, they give a much truer percentage of "breaks" than if the flower records are taken alone. Only ten bulbs of the control were grown on in the second year, and they gave a much higher percentage of "breaks" than the infected plants. On the other hand, other controls injected with distilled water (a process in which the bulbs were subjected to the same type of injury) showed no "breaks" in the second flowering season. This unsatisfactory result may justifiably be attributed to accidental secondary infection in the growing season of 1928, more especially as, in the second series of plugging experiments started in 1928, in which measures were taken to keep down aphid infestation, the controls remained normal for three years.

The "breaks" in these controls were used for grafting with parrot tulips, and the rest discarded. The treated plants were grown on till 1931, and the plugging experiments repeated with new Bartigon bulbs in the autumn of 1928 (Table VI).

#### INJECTION EXPERIMENTS OF 1927.

Bulbs injected with "broken" filtrate gave negative results in the first season after manipulation as against 40 per cent. of "breaks" in bulbs plugged with "broken" tissue. But in the second year, after a growing season with no aphid control, it will be seen that 13.7 per cent. of "breaks" is recorded. The actual controls to this experiment remained normal for two years. The three plots A, B, C, all had equal chances of becoming infected, as each plot happened to be situated in 1928 between a plot showing no "breaks" on the one side and a plot showing from 22 to 40 per cent. of "breaks" on the other. If the controls plugged with normal tissue had not "broken" heavily and some of the untreated controls likewise, this result might be taken as indicating that "breaking" was transmissible by expressed filtered sap from "broken" bulbs. All these plants were discarded in the autumn of 1929, as the results were not reliable. Meanwhile the injection experiments had been repeated in the autumn of 1928 and the results showed that, with aphid control, the treated plants remained practically normal with only one "break" in 45 plants, in the third year.

In fact all the results of the 1927 experiments cannot be relied on after the first flowering season now that we know that "breaking" can be transmitted by at least two species of aphides (14, 15); but they demonstrate very clearly how easily "breaking" can spread under ordinary



garden conditions. The records of the 1928 experiments are much more reliable, as measures were taken each flowering season to keep down aphids as far as is possible in the field.

Table III.  
*Injection experiments of 1927.*

1927  Treatment	1928			1929		
	Plants flowered	"Breaks"		Plants flowered	"Breaks"	%
A. 70 Bartigon bulbs injected "broken" filtrate	23	0	No aphid control in growing season, plants lifted and stored in autumn of 1928	31	1	3.2
B. 50 Bartigon bulbs and lateral injected "broken" filtrate	23	0		20	6	30
Total	46	0		51	7	13.7
CONTROLS:						
C. 25 Bartigon bulbs injected distilled water	9	0	Ditto (only 10 grown on)	10	0	0

#### GENERAL CONTROLS OF 1927.

Over and above controls for each individual treatment, 199 untreated bulbs were grown as a general control to the whole series of experiments. Eighty-four of these bulbs were planted as sent out by the grower; the remaining 115 had the outer brown scales removed in order that they should be subjected to the same preliminary treatment as the manipulated bulbs. The 115 bulbs were divided into four lots, three lots of 25 bulbs and one lot of 40, and the lots spaced at intervals between the treated plots.

None of these controls showed "breaks" the first year; but, as with the treated plots, they were infested with aphides during the growing season of 1928. The different plots were harvested and stored separately in the autumn of the same year. At planting time the bulbs in the bag containing plot 1 were found to be heavily infested with aphides, which, unfortunately, were not identified. The bulbs were planted just as they were without removing the aphides, with the result that 57.1 per cent. of "breaks" were recorded in the following season.

With regard to the other three peeled control plots, the correlation between the position in 1928 and the number of "breaks" in 1929 is interesting.

Table IV.  
*General controls of 1927.*

	1927	1928	1929			
Treatment		% of "breaks"	No. of plants flowered	"Breaks"	"Unbroken" plants	% of "breaks"
84 untreated controls		0	71	3	68	4.2
PEELED CONTROLS:						
Plot 1. Bulbs heavily infested with 25 aphid in storage 1928		0	14	8	6	57.1
Plot 2. No aphid in storage 25		0	20	0	20	0
Plot 3.        " 25		0	19	6	13	31.5
Plot 4.        " 40		0	25	4	21	16
Total		0	149	21	128	14

Plot 2 was situated between two plots showing no "breaks," plot 3 between a plot with 27 per cent. of "breaks" and one showing none, plot 4 between a plot with 31 per cent. of "breaks" and one showing none. The plot of 84 unpeeled bulbs was at the extreme end of the tulip ground next a wide garden path with a plot of controls showing no "breaks" on the other side.

In spite of these natural "breaks" the total percentage for the two years is low as compared with 79.7 per cent. in the grafted plots, and 32 per cent. in the plugged.

#### GRAFTING EXPERIMENTS OF 1928.

The bulb manipulation experiments of 1927 were repeated in the autumn of 1928 on a more extensive scale. A number of new bulbs of Bartigon (the same host as previously used) were bought from the same grower, but two fresh transmitters were used, Crimson Beauty and Esopus. Esopus proved to be rather a feeble transmitter as a general rule and much less potent than Crimson Beauty, except on the species *Tulipa Greigii*.

The origin of these two transmitters is not known, but they are both clear "breaks." Esopus is a taller plant and is more or less of a feathered "break"; Crimson Beauty has a shorter habit and is a less pronounced "break" with larger splashes of self-colour and rather less white ground. If it were not for the difference in habit, these two transmitters could

very well be taken for two "breaks" from the same breeder, in fact "broken" forms of the same breeder can differ very much more widely as to concentration and distribution of anthocyanin pigment than do Crimson Beauty and Esopus.

There appears to be no correlation between the type of "break" of the transmitter and the type of "break" induced. This statement only applies to the *clear* "break" and not to the *self* "break." There is no experimental evidence as yet to show whether these two types of "break" are due to different viruses or whether the *self* "break" is only an earlier stage or milder form of the more pronounced *clear* "break." Plate VI, figs. 1-5, shows the wide range of variegation in the same breeder Bartigon which can be induced by grafting with *clear* "breaks."

Three years' results of this second series of graftings are given in Table V.

Table V.  
*Grafting experiments of 1928.*

Treatment in 1928	1929		1930			1931		
	Bartigon plants flowered	"Breaks"	Bartigon plants flowered	"Breaks"	Total % of "breaks" for two years	Bartigon plants flowered	"Breaks"	Total % of "breaks" for three years
70 Bartigon grafted Crimson Beauty	48	8	58	16	27.5	59	27	45.6
70 Bartigon grafted Esopus	53	0	50	7	14	50	16	32
CONTROLS:								
50 Bartigon bulbs grafted Bartigon = 25 sets	27	0	31	0	0	39	5	12.5

The plants were kept two years as far as possible under control. They were sprayed for aphid in the growing season and lifted and stored each autumn. Esopus failed to induce "breaks" in Bartigon the first year and only 15 per cent. in the second. The controls remained normal for two years. The plants were not lifted in the summer of the second year, 1930, but were left in the ground uncontrolled during the winter of 1930-1, with the result that the percentage of "breaks" increased considerably in the treated plots and the controls showed 12.5 per cent. in 1931.

It is not possible to keep plants in the field under very strict control against insects, but the differences in the results of the years 1930 and 1931 clearly indicate that even under garden conditions, considerable

benefit can be derived from control measures in the growing season and storage in the autumn.

### PLUGGING AND INJECTION EXPERIMENTS OF 1928.

With regard to the results due to plugging in 1928 with the two new transmitters, Crimson Beauty and Esopus, little need be said; the table explains itself. It is interesting to note, however, that neither of these transmitters, which have proved all along not to be very potent, produced breaking, by means of the insertion of a small amount of tissue, in the first flowering season after manipulation. The results are, however, quite definite, although the percentage of "breaks" in Bartigon is not high. Bartigon is a variety which is held not to be a free "breaker" naturally. The controls remained normal for three years.

Table VI.

#### *Plugging and injection experiments of 1928.*

1928	1929		1930			1931			Total average for three years
Treatment in autumn of 1928	Plants flowered	"Breaks"	Plants flowered	"Breaks"	% at end of two years	Plants flowered	"Breaks"	% in the third year	
0 Bartigon plugged	19	0	32	2	6	33	6	18	33 plants in all flowered, 6 "breaks": 18 %
Crimson Beauty									
0 Bartigon plugged	21	0	24	1	4	16	4	25	24 plants in all flowered, 4 "breaks": 16.6 %
Esopus									
6 Bartigon injected	18	0	35	0	0	45	1	2.2	45 plants in all flowered, 1 "break": 2.2 %
"Broken" filtrate									
CONTROLS:									
0 Bartigon plugged	3	0	23	0	0	29	0	0	29 plants in all flowered, 0 "breaks": 0 %
Normal Bartigon									
0 Bartigon injected	3	0	23	0	0	19	0	0	23 plants in all flowered, 0 "breaks": 0 %
Normal filtrate									

The results of injections are also set out in the table, and are fairly conclusive. It was not until after the control was relaxed in the winter of 1930-1 that one "break" occurred in 45 plants.

### "BREAKING" IN KEIZERSKROON.

One exception to this infectious variegation in the bi-colour tulips has been found. The early flowering variety Keizerskroon, with a yellow margin and a fairly regular splash of red in the centre of each petal, is a true bi-colour and the variegation is not due to infection (Table VII). Bulbs of Keizerskroon grafted with normal Bartigon produce no effect, but when grafted with "broken" varieties, *clear* "breaking" is induced

in the red areas of Keizerskroon (Plate VI, figs. 7, 8). Here again Keizerskroon is more susceptible to Crimson Beauty than to Esopus.

Table VII.

*"Breaking" in Keizerskroon induced by grafting.*

Two years' results.

Variety and transmitter	No of grafts	Plants flowered	Breaks	%
Keizerskroon and Crimson Beauty	20	14	7	50
Keizerskroon and Esopus	20	16	3	18.7
CONTROLS:				
Keizerskroon and unbroken Bartigon	50	39	0	0

## GRAFTING EXPERIMENTS WITH PARROT TULIPS.

The mild form of parrotting observed in 1928 in the plots of bulbs plugged with "broken" tissue led to the surmise that the parrot tulip, with its variegated perianth and irregular margin, might possibly be some form of "break," more especially as the well-known pink variety of garden tulip Clara Butt (a variety which "breaks" naturally very freely) has been known to throw a handsome parrot sport "Phantasy" within recent years.

In October, 1928, 100 normal Clara Butt and 80 normal Bartigon were grafted with five different varieties of parrot tulip.

Such a pronounced morphological change from an entire to a lobed or toothed margin of the perianth could hardly be expected in one season, as the flower in the tulip is fully laid down at a very early stage, and the meiotic divisions in the pollen mother cells are known to take place as early as September in some species and garden varieties. As the bulbs were treated at the end of September, the anthers and other parts of the flower must have been fully differentiated before that time. The results were entirely negative throughout.

It was then thought that perhaps "breaking" was a necessary intermediate step towards parrotting, and also that the size and maturity of the host bulbs when manipulated might be factors to be considered. In October, 1929, a number of Bartigon bulbs of induced and natural "breaks," varying in size and maturity, were grafted with the same five varieties of parrots, and over and above these, 29 flowering bulbs of naturally "broken" Clara Butt were grafted with two new varieties of parrots, Large Yellow and Fire King.

The results of the two series of grafting experiments are set out in Table VIII. The first year two plants in plot C and six in plot E showed

abnormalities; in the second year these abnormalities disappeared entirely and the results were again negative.

Table VIII.

*Grafting experiments with parrot tulips.*

Treatment	1928	1929			1930			Total for two years		
		Plants flowered	Normal flowers	Abnormal flowers	Plants flowered	Normal flowers	Abnormal flowers	Plants flowered	Normal flowers	Abnormal flowers
<b>NORMAL:</b>										
A. 80 normal Bartigon grafted 5 vars. of parrots		71	71	0	72	72	0	72	72	0
B. 100 normal Clara Butt grafted 5 vars. of parrots		81	81	0	74	74	0	81	81	0
<b>INDUCED "BREAKS":</b>										
		1930			1931			Total for two years		
C. 133 "broken" Bartigon grafted 5 vars. of parrots		80	78	2	86	86	0	86	84	2
<b>NATURAL "BREAKS":</b>										
D. 34 "broken" Bartigon grafted 5 vars. of parrots		17	17	0	14	14	0	17	17	0
E. 29 "broken" Clara Butt grafted 2 vars. of parrots		27	21	6	17	17	0	21	15	6
376 Total		276	268	8	263	263	0	277	269	8

The number of Bartigon plants which flowered in plots C and D is rather low, as might be expected, as in these two plots the host bulbs varied considerably in size and maturity when manipulated, some of them being much too small to contain a flower bud. The number of plants which survived the treatment is surprisingly high, however, and the plants will be grown on again in 1932.

A number of bulbs were also plugged with parrot tissue, but so many treated bulbs failed that the results are not worth recording.

INDUCED "BREAKING" IN *TULIPA* SPECIES.

Two species of *Tulipa*, *T. Greigii* and *T. Eichleri*, and the somewhat doubtful species *T. gesneriana spathulata*, were grafted in the autumn of 1928 with Crimson Beauty and Esopus. On account of expense only a small number of bulbs could be treated, but the results show quite definitely that the same virus which causes "breaking" in the garden tulip can be transmitted to the species and bring about "breaking." The type of "break" induced in the species, however, is a *self* "break," although both the transmitters were *clear* "breaks" (Plate VII, figs. 1-3). Crimson Beauty proved to be more potent on *T. Eichleri* than on

*T. Greigii* and the reverse is the case with *Esopus*. *T. gesneriana spathulata* showed exactly the same percentage of "breaks" with both transmitters, and the controls remained normal for two years.

Table IX.

Grafting experiments with *Tulipa* species.

1928	1929		1930		Total for two years		
	Plants flowered	"Breaks"	Plants flowered	"Breaks"	Plants flowered	"Breaks"	Total % two years
Species and transmitter							
9 <i>T. Greigii</i> grafted	8	1	No further		8	1	12.5
Crimson Beauty			"breaks"				
9 <i>T. Greigii</i> grafted	7	3	Ditto		7	3	42.8
<i>Esopus</i>							
9 <i>T. Eichleri</i> grafted	6	4	Ditto		6	4	66.6
Crimson Beauty							
9 <i>T. Eichleri</i> grafted	6	1	Ditto		6	1	16.6
<i>Esopus</i>							
21 <i>T. gesneriana spathulata</i> grafted	19	0	12	3	19	3	15.7
Crimson Beauty							
20 <i>T. gesneriana spathulata</i> grafted	19	0	11	3	19	3	15.7
<i>Esopus</i>							
CONTROLS FOR TWO YEARS:							
6 <i>T. Greigii</i>	6	0	—	—	—	—	—
6 <i>T. Eichleri</i>	6	0	—	—	—	—	—
10 <i>T. gesneriana spathulata</i>	10	0	—	—	—	—	—

*Tulipa* species, as far as these experiments go, do not show the same tolerance to the virus as the garden varieties. The flowers are mostly smaller and the margins of the petals inclined to be irregular and sometimes narrower. But the controls also showed signs of loss of vigour under garden conditions, so that this more marked pathological condition in "broken" species may only be the result of environment.

DEGREES OF POTENCY OF TRANSMITTERS AND  
SUSCEPTIBILITY OF HOSTS.

Climatic conditions may have something to do with the intensity of "breaking," but the results obtained (Table X) from the two transmitters, Crimson Beauty and *Esopus*, subjected to the same conditions in the same season, clearly show that any two transmitters can differ in degree of potency, and that the same host can show varying degrees of susceptibility towards different transmitters. If it were not for the exceptional case of *T. Greigii*, *Esopus* might be looked upon as a weaker transmitter than Crimson Beauty; but the lowest percentage in the table is from Crimson Beauty grafted with *T. Greigii*. This shows that

this particular species is not unduly susceptible to "breaking" in general. It is interesting also that both transmitters are equally potent on *T. gesneriana spathulata*, and that the latter is not a very susceptible species. Keizerskroon and *T. Eichleri* appear to be the most susceptible. Both transmitters are *clear* "breaks"; the "breaks" induced in garden varieties were also all *clear* "breaks," but those induced in the species tested were all *self*.

Table X.

*Showing degrees of potency of transmitters and susceptibility of hosts.*

Host grafted	Records for two years under control.							
	Transmitter, Crimson Beauty				Transmitter, Esopus			
	No. of grafts	Plants flowered	"Breaks"	%	No. of grafts	Plants flowered	"Breaks"	%
Bartigon	70	58	16	27	70	50	7	14
Keizerskroon	20	14	7	50	20	16	3	18.7
<i>T. Greigii</i>	9	8	1	12.5	9	7	3	42.8
<i>T. Eichleri</i>	9	6	4	66.6	9	6	1	16.6
<i>T. gesneriana spathulata</i>	21	19	3	15.7	20	19	3	16.6

#### PIGMENT IN THE TULIP.

The red and purple pigments in the tulip are anthocyanin sap pigments, entirely confined, as far as the perianth is concerned, to the epidermal layer.

The yellows are mostly plastid colour, although some yellow varieties are said to have a yellow flavone (*i.e.* sap pigment). Yellow plastids can occur both in the epidermis and the mesophyll. When anthocyanin is present in the flower, the epidermal plastids can occur with anthocyanin in the same cell; or, as is more usual, the plastids are found in scattered cells or groups of cells devoid of anthocyanin. The epidermal plastids are of two kinds; large well-defined bodies of a deep yellow colour resembling chloroplasts except for the colour, and very small elongate-oblong bodies of a diffuse yellow colour. It is not known whether these smaller bodies are merely the result of the disintegration of the larger plastids, but they are mostly segregated in different cells.

These two forms of plastids can easily be distinguished from the mesophyll plastids; the latter are quite well defined but smaller and of a paler colour than the larger epidermal plastids.



As regards pigment, the tulips can be roughly classified as follows:

1. Pure colours. A continuous layer of anthocyanin over a mesophyll devoid of yellow plastids; pinks, crimson, mauve, and purple.

2. Mixed colours:

(a) A continuous layer of anthocyanin over a mesophyll containing yellow plastids; orange, scarlet, red, browns.

(b) Non-continuous layer of anthocyanin (which looks continuous to the naked eye) with some cells containing both sap pigment and yellow plastids, together with other scattered cells or groups of cells containing one or both forms of epidermal plastids but devoid of anthocyanin, over a mesophyll with or without yellow plastids; orange, scarlet, bright crimson, browns and various art shades.

3. Yellows. No anthocyanin sap pigment, plastids in the epidermis and mesophyll, and possibly a yellow flavone.

4. Whites. No anthocyanin and no yellow plastids.

The pure colours "break" to a white ground; the mixed to a yellow ground in 2 (a) and to yellow, cream or white in 2 (b) according to whether the mesophyll and epidermal cells contain yellow plastids or not.

*Self* "breaks" can occur in both 1 and 2.

"Breaking" in a flower of a pure yellow variety is rather indefinite, taking the form of white semi-transparent, or paler areas on the self-coloured ground. The leaves, however, can show typical "breaking." Although it is highly probable, there is, as yet, no experimental evidence that this somewhat indefinite irregularity represents a true flower "break." Grafting experiments with a yellow variety of garden tulip, Mrs Moon, have given very indefinite results. The general percentage of "breaks" was low and there were more "breaks" in the control than in the treated plots.

In the parrot tulips it is impossible to detect "breaking" in the flower with any degree of certainty, on account of the irregular distribution of pigment natural to these tulips, but the leaves can show typical "breaking" in infected plants. There is no hard and fast distinction between many of the named varieties of parrots which are mainly yellow in colour, they merge into one another. This graduation may be due, in part, to some plants being infected with the "breaking" virus, and by analogy with what is now known to happen in "broken" Keizerskroon it is probable that only the areas containing anthocyanin would be affected. With varieties such as Constantinople and Cramoisie Brilliant (two of the parrot transmitters used in these experiments), in which the anthocyanin is fairly evenly distributed over a considerable

area of the perianth, it is possible to detect *clear* "breaking" in the flower of a plant showing "broken" leaves.

In a "broken" flower containing anthocyanin there is no gradual transition in colour from a normal self-coloured cell to one containing more concentrated anthocyanin or to one devoid of sap pigment. The change is abrupt, and darker, normal, and colourless cells can lie in contact with one another.

#### DISCUSSION AND GENERAL CONCLUSIONS.

The virus in the tulip can do three things simultaneously in the same plant; it can affect the chloroplasts in the leaves, destroy or inhibit the formation of anthocyanin in some areas and bring about a higher concentration of presumably the same pigment in other areas of the same flower. Sharply delimited patches of concentrated pigment occur both in the decolorised areas and in areas in which the original self colour has not been affected.

There are three possible explanations for this marked difference in colour and degree of concentration between individual cells or group of cells which, in all other respects, appear to be identical. The difference may be due to infection with more than one virus, or only a matter of degree of infection, or it may be that the cells are physiologically different and react differently towards the same virus.

During the first few years after a *clear* has appeared in a breeder plant, the colour distribution or pattern varies somewhat from season to season, and the plant may produce "unbroken" laterals. This is more especially the case when the first "break" is of a mild form. A mild *clear* "break" usually increases in intensity in subsequent seasons (*i.e.* larger areas are decolorised) until a limit is reached, when the type of "break" becomes stabilised, and from henceforth varies only within narrow limits. For instance, a plant with a stabilised feathered "break", continues to show more or less the same type and amount of feathering year after year. This suggests that the cells of a flower differ physiologically and that each bulb has its own individual basic pattern. But it does not necessarily follow that all the "breaks" from the same variety of breeder will always throw the same type "break," as can be seen in Plate VI, figs. 1-5.

There are numerous records of sports or sudden mutations in the tulip. These marked and sudden changes throw light on the question as to how a breeder variety, derived in the first instance from a single seedling and increased by vegetative propagation, can produce such a

wide range of pattern. It is quite possible that this wide range of "break" pattern may be due to smaller and less pronounced bud mutations, more especially in a plant such as the tulip. It has been crossed so often and over such a long period of time that the present-day varieties must be of very mixed origin.

With regard to the effect of the virus on the pigment plastids, very little can be said until a much larger number of pure yellows and *clear* yellow "breaks" in flowers containing anthocyanin have been examined. Generally speaking, the mesophyll plastids in flowers containing anthocyanin are not as a rule affected. In flowers devoid of anthocyanin, in the yellow variety Mrs Moon for example, it is known that plastids occur in the mesophyll and two kinds of yellow plastids in the epidermal cells; but in the white areas of the "broken" flower no plastids are to be found.

In the green bud of the tulip, chloroplasts are present in the mesophyll; but, with the exception of the guard cells, there are no chloroplasts or pigment plastids in the epidermis. The yellow plastids in the epidermis develop as the flower colours and must be true pigment plastids. It is not known at present whether the yellow mesophyll plastids are ageing forms of the chloroplasts or not; in all probability they are not, as, in the pure coloured tulips, no trace of mesophyll plastids can be found in the full-blown flower.

A knowledge of the translocation of the virus in the tulip plant may be of some practical importance. The tulip bulb does not persist from year to year as in the hyacinth or narcissus. It is replaced each season by a new bulb, generally formed at the base of the current year's flowering shoot. The bulb of a self-coloured breeder, as the name implies, proliferates very freely, and it is quite usual for two new bulbs of flowering size and several fair-sized laterals to develop from a single "unbroken" breeder bulb in one season. Once "broken" the bulb does not proliferate so freely. The laterals arise from buds which have developed the previous season in the axils of the outer scales of the parent bulb, remote from the main growing point. In the second season's growth, these laterals can throw out roots of their own, and although sometimes still quite small, can carry on a more or less independent existence.

The elaborated food substances passing down from the green parts of the plant cannot be translocated laterally from scale to scale in the bulb, but have to reach the base before they can be distributed to the regions where active growth is going on, such as the buds in the axils

of the scales, etc. In the early stages of spring growth this base is intact, but by the time the plant has come into flower the new bulb has attained a considerable size and the base and scales of the old bulb have shrivelled. Caldwell(7) has been able to show that the virus of the aucuba mosaic of tomato and tobacco cannot pass through or out of a dead cell. The same is probably true of the tulip virus. If infection, by means of aphid vectors, takes place through the leaves in the growing season, the virus can reach the new main bulb, still in open communication with the flowering shoot, but cannot pass across the dead tissues of the base and reach the remoter laterals, and the latter remain uninfected. Hence, if a valuable new seedling "breaks," instead of discarding the whole plant, it is worth while saving the remoter laterals, as they may have escaped infection, and if grown on separately under aphid-free conditions may remain "unbroken." On the other hand, however, since the virus can reach the buds which form the same season inside the new main bulb at the base of the freshly "broken" flowering shoot, the laterals from that bulb will show "breaking" in subsequent seasons.

A number of sets of bulbils and laterals saved from individual recent "breaks" (both natural and induced) have been under observation for the last three years. The main bulbs of these initial "breaks" were discarded or used for further grafting experiments with parrot tulips; but the laterals and small bulbils were grown on in separate sets in pots sunk in the soil. Also some complete sets (including the main bulb) from plugged bulbs which had not shown "breaking" the first year, were planted in the same way.

In some sets all the flowers were normal and remained normal for three years; in others all the flowers showed much the same type of "break" in the second year and remained "broken," or some only showed "breaking" in the third year; but in a certain number of cases both "broken" and normal flowers appeared in the same set. There are also a few records of apparent recovery in the sets from bulbs plugged in 1927. In the first year a slight "break" was induced, but in the second and third years these sets showed no "breaking."

The process of plugging which entails cutting diagonally or transversely across the scales on one side of the bulb causes so much damage that, in the majority of cases, the plugged side of the bulb rots away early in the growing season, with the result that only the outer laterals and smaller bulbils remote from the plug survive. The laterals need not necessarily become infected for the reasons already given, and may not flower until the second or third season after harvesting. When the damage

is extreme, the whole bulb rots and no plant appears above ground; but when the remains of the old bulb can be found in the soil it is sometimes possible to secure small bulbils from the rotting tissues. When the damage is not so extreme the treated bulb may flower the first year after treatment, but is too much weakened to produce a flowering bulb for the next season, with the result that again only the laterals remote from the plug can develop and may escape infection, but will not flower until the second year. Therefore those cases in which "breaking" disappeared in the second year cannot be looked upon as real recoveries.

Unfortunately the exact position of the various laterals and bulbils were not recorded when harvesting these initial "breaks," as the experiment was not devised for this purpose, and the importance of this point was not realised. The results are therefore given for what they are worth and no claim is made that they are conclusive.

#### SUMMARY.

The results of the second series of bulb manipulation experiments have confirmed previous results as to the infectious nature of the agent or virus which brings about "breaking" in tulips.

"Breaking" can be transmitted by grafting and plugging bulbs with tissue from "broken" bulbs, but injections with filtered sap expressed from "broken" bulbs have given negative results.

All attempts to induce parrotting by grafting have been unsuccessful.

There appears to be no correlation between the type of "break" of the transmitter and the type of "break" induced.

Transmitters vary in potency on different hosts, and different hosts vary in degree of susceptibility towards the same transmitter.

*Tulipa* species have been observed to "break" naturally under garden conditions, and "breaking" has also been induced in the same by grafting with "broken" garden varieties.

The bi-colour variety Keizerskroon has been proved not to be an infectious "break," but a true bi-colour; "breaking," however, can be induced in the red areas of the perianth by grafting with other "broken" varieties.

The effect of the virus on the colour plastids and the distribution of the anthocyanin sap pigment is discussed.

It is suggested that a knowledge of the translocation of the virus in the bulb is of practical importance to bulb growers.

The experiments indicate the importance of aphid control by means

of spraying in the growing season and the advantage to be gained by lifting and storage under aphid-free conditions in the autumn.

In conclusion, my thanks are due to Sir D. Hall for information with respect to the initial "breaking" of Zomerschoon and the "breaks" in *Tulipa* species, and to the laboratory assistant E. F. Emarton for taking the photographs for the plates and for his valuable help in the manipulation of the large number of bulbs treated.

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## EXPLANATION OF PLATES VI, VII.

## PLATE VI.

- Figs. 1-5. Different degrees of *clear* "breaking" induced by bulb manipulation in the same breeder, Bartigon.  
Fig. 6. Normal Bartigon.  
Fig. 7. Normal Keizerskroon.  
Fig. 8. Induced *clear* "break" in Keizerskroon.  
Fig. 9. Normal leaf of *Tulipa Eichleri*.  
Fig. 10. "Broken" leaf of *Tulipa Eichleri* induced by grafting.

## PLATE VII.

- Fig. 1. Three flowers of *Tulipa Eichleri*: (a) and (c) induced *self* "breaks," (b) normal flower.  
Fig. 2. Three flowers of *T. Greigii*: (b and (c) induced *self* "breaks," (a) normal flower.  
Fig. 3. Three flowers of *T. gesneriana spathulata*: (b) and (c) induced *self* "breaks," (a) normal flower.

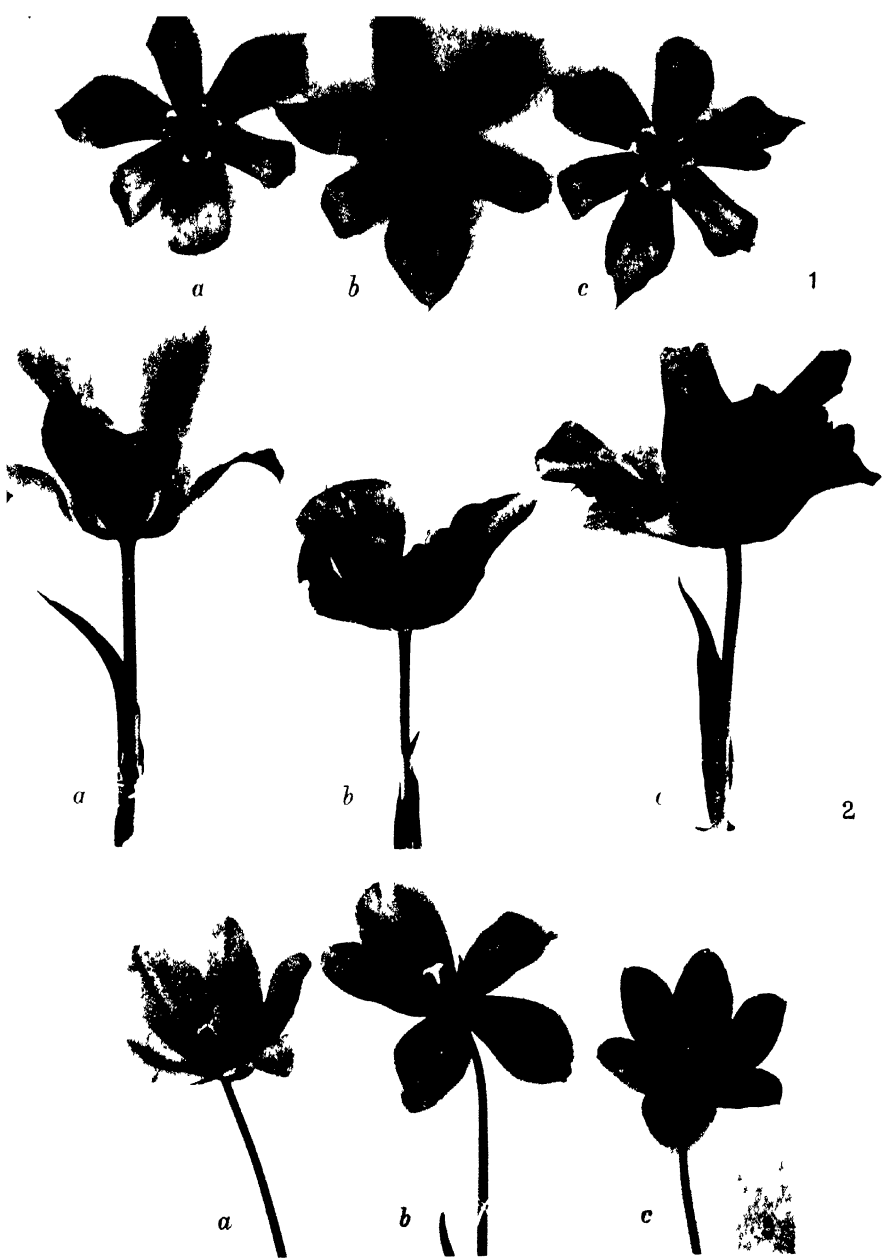
(Received September 1st, 1931.)



CAYLEY.—“BREAKING” IN TULIPS. II (pp. 153-172).









## A BARK DISEASE OF COFFEE IN EAST AFRICA

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(With Plates VIII and IX and 1 Text-figure.)

A DISEASE of the bark of young leader shoots of *arabica* coffee occurs in the plantations of the Usambara Mountains of Tanganyika Territory. The disease culminates in the ringing and death of the shoot. The death of such shoots is usually of no importance, since they would be removed in the normal process of pruning. Where, however, the procedure of cutting old bushes down to stumps 1–2 ft. high is adopted in an attempt to regenerate old plantations, a disease of the shoots which arise from the stumps is a matter of serious concern. The report of the death of large numbers of such stumped coffee plants on an estate in the West Usambaras led to this investigation.

I have been able to show that this disease is due to the invasion of the tissues by *Fusarium lateritium* Nees var. *longum* Wr. The fungus has not hitherto been recognised as a pathogen of coffee. Bark disease of coffee associated with species of *Fusarium* has been reported by Fawcett<sup>(2)</sup> in Porto Rico, and by Toro<sup>(5)</sup> in Colombia, but without specific determinations or positive evidence from inoculations. The disease has not been previously recorded in East Africa.

These investigations have been carried out at the East African Agricultural Research Station, Amani, and at Sakarre Estate, to the management of which, and to Commander H. V. Low in particular, I owe my thanks.

### OCCURRENCE.

This disease is known to have occasioned serious economic loss only in one plantation on Sakarre Estate in the West Usambaras. The bushes of this plantation, reputed to be some thirty years old, were stumped in 1927. The shoots from these stumps, which at first grew strongly, began to die off in various stages of growth; some when only a few inches long, others only after growing to a height of 4–5 ft. By 1930 the majority of the plants in one part of the plantation were affected, and many, having produced a succession of shoots, were themselves dead.

In this plantation I observed also young seedling plants which had died with the same symptoms. A characteristic spot on the leaves of young seedlings was shown to be caused by the same pathogen. Elsewhere in this district I found affected sucker shoots arising from the main stems of otherwise healthy bushes.

In the East Usambaras the disease occurs sporadically in all plantations visited, but at present causes insignificant loss.

#### SYMPTOMS AND SIGNS.

The disease of the stem is characterised by lesions in the extra-cambial tissue. In a green stem the young lesion is readily seen as a slightly shrunken area, with a "water-soaked" margin, varying in colour from cinnamon (15") to tawny olive (17" *i*)<sup>1</sup> (Plate IX, fig. 6). At a later stage the lesion darkens in colour, varying from almost black to bister or snuff brown (15" *m* and *k*) with sometimes lighter tones in the centre. An orange "halo" usually grades into the surrounding green of the stem (Plate IX, fig. 8).

On a bark-covered shoot the bark covering the lesion usually remains intact, so that the lesion may be hardly discernible without cutting open to expose the dead brown tissue. Generally, however, the lesion appears shrunken, as a result probably of growth of the surrounding stem tissue. In consequence a characteristic feature of the advanced stage of the disease is a pronounced constriction in the stem (Plate VIII, fig. 1). Externally the margin of the lesion is usually not sharply marked but grades smoothly into the surrounding healthy tissue. Rarely, however, a swollen ridge is evident at the margin, and in this canker form of lesion the bark tends to shred off and leave the wood exposed.

The lesion in the stem of a shoot is usually located within a few inches of the base of the shoot. After the death of the shoot the lesion may spread down into the tissues of the stump upon which the shoot was growing. Such spread appears not to take place into the main stem of a growing bush when a sucker shoot, borne by it, dies from this disease.

The enlargement of the lesion results usually in the ringing of the stem and eventually in the death of the whole shoot. Many months may elapse, however, before the leaves of the shoot wilt. Generally the younger the shoot, the sooner it succumbs. Thus, in a series of observations made upon young green shoots in the plantation, the period from

<sup>1</sup> The colour nomenclature is that of Ridgeway (*Colour Standards and Colour Nomenclature*, Washington, D.C., 1912, 43 pp.). The numbers quoted refer to the standards in that book.

the date when noted as healthy to that when noted as dead varied from 28 to 240 days. In experimental inoculations, seedlings with 4-5 pairs of leaves became ringed and wilted within about a month from the first appearance of the lesion. In shoots 2-3 ft. high the lesion required from 3 to 9 months to ring the stem. The wilting of these shoots usually followed within 1 or 2 months after ringing; but in some instances they retained many of their leaves, which, however, were yellow and sickly, and even produced flowers and new leaves for as long as 9 to 18 months after ringing. In older bark-covered shoots in the plantation the disease was occasionally observed to run its full course within 1 month; usually, however, the lesion required several months to pass around the stem and thereafter shoots frequently survived for more than a year before their foliage wilted. Consequently I commonly found in the plantation shoots which were completely ringed, yet bore apparently normal foliage.

Wilting, when it eventually occurs, is sudden, and the leaves hang dead and brown upon the dead shoot for a considerable period (Plate IX, fig. 5).

Histologically, in the lesion all tissues down to, and including, the cambium are dead. Beyond the margins of an advancing lesion no obvious reaction by the plant cells is to be seen. Cambial activity appears to proceed normally. In certain instances, however, where the advance appears to have been checked, a callus growth beyond the margin is evident, tearing up the adjacent dead tissue from the wood. These differences in the behaviour of the cambium are reflected in the differing external appearance of the two types of lesions already described.

The leaf spots, caused by the same organism as the stem lesions, are circular or irregular in outline, but with rounded margins, up to 14 mm. in diameter (Plate IX, fig. 7). Their colour is, on the upper surface, warm sepia (13" *m*) with a partial "overlay" of lighter tones in irregular concentric zones; on the lower surface, snuff brown (15" *k*) and tones of 15". The edge of an old lesion may be sharply defined by a narrow translucent line, outside which is a diffuse yellow halo grading into the green of the leaf. Histologically this translucent margin is seen to consist of typical wound cork, formed from the mesophyll cells in the manner described by Butler<sup>(1)</sup>.

Under favourable conditions the lesions on stems and leaves bear pink sporodochia of *Fusarium* spores. Internally they are permeated by hyphae varying in diameter from 1.5 to 4.5  $\mu$ , intercellular except in the middle of an old lesion. Hyphae have not been found in the wood beneath a stem lesion.

## ETIOLOGY.

*Isolation of fungi.* Platings of pieces of tissue taken from the margins of lesions and externally sterilised gave either no growth—due probably to too severe sterilisation—or a fungus growth of one of four types, A 1 (seven times), A 2 (twice), B (four times) and C (five times). Cultures from single spore isolations of these fungi have been determined by Wollenweber<sup>1</sup> as follows:

A 1. *Fusarium lateritium* Nees var. *longum* Wr. (*Fusaria autographice delineata*, No. 964).

Conidia, from sporodochia on natural substratum: 5 septa dominant; 3, 4 septa frequent; 1, 2, 6, 7 septa rare.

Sizes of conidia: 5 septate,  $45\text{--}71\mu \times 4\text{--}5\cdot5\mu$ ; 4 septate,  $40\text{--}54\mu \times 4\text{--}5\mu$ ; 3 septate,  $24\text{--}52\mu \times 3\cdot7\text{--}4\cdot5\mu$ .

Brief cultural characters: aerial mycelium white to pink, occasionally buff, but character of producing mycelium soon lost in culture. Spores produced sparsely in minute sporodochia on surface of agar slant (corn-meal, potato dextrose). Minute black sclerotia in agar. Develops a deep carmine (1 i) colour in agar (corn-meal, potato dextrose, prune) and in steamed rice. This species is reputed to be the imperfect form of *Gibberella baccata* (Wallr.) Sacc.

(Wollenweber, *Angew. Botanik.* VI, 300). I have not obtained perithecia in my cultures.

A 2. As A 1.

Conidia as above.

Differs from A 1 in producing sclerotia more freely and in developing faint dusky blue green (39" m) in agar. After culture for some months this strain started to produce carmine coloration faintly.

B. *Fusarium eumartii* Carpenter (*Fusaria autographice delineata*, No. 422).

<sup>1</sup> I acknowledge my debt to Dr Wollenweber of the Biologische Reichsanstalt, Berlin-Dahlem, and to Dr S. F. Ashby, of the Imperial Mycological Institute, through whose hands my cultures passed.

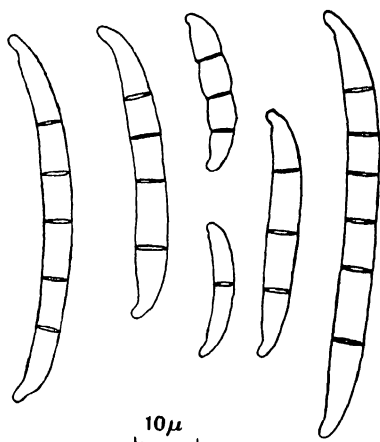


Fig. 1.

*C. Gloeosporium coffeicola* (P. Henn.) Wr. (Syn. *Fusarium coffeicola* P. Henn.), (*Fusarium autographice delineata*, No. 493).

I judge this fungus to be identical with that known generally in East Africa as *Colletotrichum coffeanum* Noack. (*Zeit. Pflanzenkr.* xi, 202). I have observed setae rarely in acervuli on coffee stems from which this fungus has been isolated.

*Inoculations.* Preliminary inoculations of portions of tissue from fresh stem lesions into cuts in the green stems of twenty *arabica* seedlings, about 2½–3 ft. high, produced lesions in all but one. In eight plants the lesion passed around the stem and resulted eventually in the death of the upper part of the plants. One plant, however, survived for over 18 months after being completely ringed; at the end of this period the constriction at the point of inoculation was pronounced (Plate VIII, fig. 2). The remaining inoculated plants, after a considerable development of the lesions, formed a callus at the margin and the lesion ceased to advance (Plate VIII, fig. 3).

Inoculations of pure cultures of the four fungi isolated—obtained by the dilution plate method from spores formed in culture—were made into leaves and stems. All inoculations of leaves without wounding were unsuccessful. Inoculations of cultures A 1 and A 2 into needle punctures made in leaves of young seedlings usually resulted in definite lesions similar to those seen in the field (Plate IX, fig. 7); inoculations of B and C resulted in no more than a slight browning of the margin of the wound, indistinguishable from that produced in control inoculations with sterile water.

Young green shoots growing from stumped *arabica* plants in a plantation at Amani were inoculated with pure cultures of the four fungi isolated. When agar bearing mycelium and spores was introduced into cuts in the stems—subsequently bound with tin-foil—definite lesions resulted in all of seven inoculations of A 1 and of seven inoculations of A 2; in none of seven inoculations of B and in none of three inoculations of C. Of the fourteen lesions so produced, two only eventually ringed and killed the shoots, the remainder healing with a callused margin. Inoculations of emulsions of spores from pure cultures, introduced into the cortical tissue of the stem by means of a hypodermic needle, resulted in lesions in thirteen out of sixteen inoculations of A 1 and in all of seven inoculations of A 2 (Plate IX, fig. 8). Of these twenty lesions six eventually ringed the stems and the shoots died. Of ten similar inoculations of B, one only formed a small lesion. In all the preceding experiments parallel control inoculations of sterile agar or



sterile water gave no lesions, the wounds healing with a trace of browning at the margins.

Older shoots, with the stems barked over, were inoculated with pure cultures into cuts. Lesions resulted from the inoculation of A 1 and A 2, while none resulted from inoculations of B or sterile agar. In all instances the lesions healed with a callused margin. These inoculation experiments were carried out with identical results both at Amani and in the plantation at Sakarre where the disease was first discovered.

The fungi inoculated were successfully reisolated from the margins of lesions produced in the foregoing experiments—A 1 three times and A 2 four times. When reisolated these two strains of the one species retained the cultural characters which had served to separate them in the first instance.

Experiments were carried out in the inoculation of the fungi in pairs into the leaves and stems of young *arabica* plants. Inoculations of A 1 or A 2 gave lesions no more active than those produced by either culture alone or by either in combination with B or C. Cultures B and C together gave no lesions.

These experiments demonstrated that *Fusarium lateritium* var. *longum* is pathogenic to the leaves and stems of *arabica* coffee. No difference was observed in the pathogenicity of the A 1 and A 2 strains. *Fusarium eumartii*, although isolated several times from natural lesions, appears to be unable to initiate an attack on the plant. *Colletotrichum coffeanum* is generally regarded as a weak parasite of coffee (4), although one strain is an active parasite of coffee berries in Kenya (3). It has appeared invariably when I have made isolations from the newly formed bark of healthy shoots. I judge this species to be usually the first invader of the cortical tissues of the stem when these are cut off by a deep-seated cork cambium in the normal process of growth. The frequent occurrence of this species in isolations made from lesions of the disease considered here is therefore sufficiently explained.

It may be questioned whether I have advanced sufficient evidence for the view that the naturally occurring disease is due to *F. lateritium*; that is, whether the experimental disease is identical with the natural disease. Whereas in the field the disease usually ends fatally and recoveries are rare, a large proportion of the experimentally diseased plants recovered. In particular, in none of the old shoots which were inoculated did the lesion pass around the shoot and kill it.

The field evidence, however, allows of little doubt that the natural disease in young shoots is identical with that occurring in adjacent old

shoots. The disease was present in shoots of all ages up to the largest. The course of the disease was identical in all such shoots. Spread traced through the stump tissues from a lesion on an old shoot resulted in the death of young shoots. *F. lateritium* was isolated from lesions on young and old shoots. The inoculation of tissue from old shoots resulted in typical lesions on young shoots.

I have successfully produced by inoculation lesions upon young shoots identical with those observed on similar shoots in the field. The frequency of recovery in my experimental plants I attribute to a failure on my part to provide exactly suitable conditions for the full development of the disease. That the physiological state of the plant exerts a large influence on this disease is suggested by the extreme localisation of the disease in one portion of one plantation. The failure of my inoculations of large shoots in this plantation is, however, not easily explained. But it is possible that, in choosing suitable plants for inoculation, I selected plants which had escaped the natural disease and might therefore be expected to show some resistance to it.

Furthermore, naturally recovered shoots are not unknown in the plantation, and show similar features to the inoculated shoots.

#### THE MANNER OF NATURAL ENTRY OF THE FUNGUS INTO THE PLANT.

I have already mentioned that the fungus may pass down a shoot killed by it into the tissues of a stump. Within the stump tissues spread may occur, until the base of another shoot is reached. Consequently the death of a shoot on a stump is often due to an invasion by the fungus passing from the stump. Lesions formed in this way have been repeatedly found in the field, and a clear connection of discoloured stump tissue traced to the base of an old dead shoot. In certain of my experimentally inoculated plants I have observed a similar spread to adjacent shoots.

The majority of the lesions seen in the field, however, are completely surrounded by healthy tissue and have clearly originated as the result of a natural inoculation of the fungus. Doubtless spores carried by wind or otherwise have initiated a primary attack at this point.

I have endeavoured to discover the manner of entry of the fungus into unwounded plants. A study of young lesions in the field showed that while a few lesions had originated at mechanical wounds, and a few were confined to a single internode, the great majority were more or less centred upon a leaf scar. Rarely a dead leaf remained still attached.

The failure of attempts to inoculate seedlings experimentally without wounding has been already noted. The manner of entry of the fungus into leaves therefore remains obscure. However, following up the observations described, I performed experiments to decide whether, given a successful leaf infection, the fungus could pass down from the leaf into the stem. The fungus was inoculated into wounds in the base of leaves of young seedlings. Seven series of plants of various ages up to 2 ft. high, were inoculated, involving in all forty-four plants. In two instances the fungus, after forming a typical spot on the leaf, passed down the petiole and a lesion formed in the stem, spreading from the base of the leaf. The dead leaf remained attached in the centre of the lesion. In almost every other instance a leaf-spot followed inoculation, but within 5-12 days, before the spot had spread down the petiole, the leaf was shed naturally and no invasion of the stem followed. The control leaves, similarly inoculated with sterile agar only, were not shed, the wounds made in them healing without discoloration of the tissues.

The fresh leaf-scar exposed by the shedding of the leaves was inoculated by placing upon it mycelium and spores of A 1. Care was taken to avoid wounding the exposed surface and to ensure that the leaf fell of its own weight and was not torn off prematurely. A moist atmosphere was maintained by bell-jars placed over the plants. Lesions resulted in three out of five plants. These lesions spread at first down the leaf-base and into the interpetiolar stipules and eventually in two plants ringed the stems at and below the inoculated node. Four control plants, inoculated with sterile agar upon the fresh leaf-scars, did not develop lesions.

In a repetition of this experiment on identical lines, all of twelve plants formed lesions at the inoculated leaf-scars and eventually became ringed (Plate VIII, fig. 4).

In the preceding experiments the inoculum was placed on the leaf-scar within 24 hours of the shedding of the leaf. A series of inoculations made on leaf scars, which had been exposed for 2-24 days, resulted in no infections. The conditions under which this experiment was performed were identical with these of the successful experiment described in the preceding paragraph.

A further experiment, in which the inoculum consisted of a spore emulsion only, resulted in lesions in three plants out of six inoculated. Control plants, inoculated similarly with distilled water, remained healthy.

From the results of these experiments I deduce that many of the natural lesions in the field are the consequence of invasion by the fungus

through leaf-scars. It is probable that *F. lateritium* may take only a minor part in causing the actual leaf-fall; many agents cause premature leaf-fall in the plantation, including probably the most important agent, *Hemileia vastatrix*, the fungus of leaf-rust. Possibly also the scars exposed by natural leaf-fall at the maturity of the leaf may not bar the entry of the fungus. It may be noted that Wiltshire<sup>(6)</sup> demonstrated that infection by the apple canker fungus (*Nectria ditissima* Tul. (*N. galligena* Bres.)) may be initiated at a leaf-scar.

The evidence that a number of lesions in the field appeared to originate almost at the junction of the shoot with the stump suggested that, in forcing its way out through bark of the stump, the bud of the young shoot might open a channel to the fungus. This was tested experimentally by placing mycelium and spores of A 1 upon the swelling buds of four stumps. No infection resulted, however, until the shoots had grown to a length of about 6 in. Lesions then appeared on many of the shoots, and their position suggested that the fungus had entered through small basal scale leaves.

The manner of natural invasion of shoots, which have barked over, is not known. It is certain that lesions may develop on shoots up to  $1\frac{1}{2}$  in. in diameter. I am uncertain, however, whether these lesions are the result of the slow development of an infection which started before the bark formed, or whether the natural inoculation of the fungus occurred after bark formation. In a series of old shoots kept under observation, lesions appeared in shoots which at the first examination were undoubtedly healthy. But the examination necessarily involved some cutting of the bark, and the fungus may have entered through the wounds so caused.

#### THE SUSCEPTIBILITY OF COFFEE SPECIES.

The disease is primarily one of *arabica* coffee, upon which all the experiments hitherto described have been performed. In one series of experiments half of the plants were the Kent variety of *arabica*; they showed, however, a susceptibility about equal to that of *arabica*.

Inoculations of *C. liberica*, *robusta* and closely related types (*C. quillou*, *canephora* and "*Congensis* hybrid"<sup>1</sup>), in comparison with parallel inoculations of *C. arabica*, indicated a high resistance in those species. Small lesions, which quickly healed, developed in *liberica*, *canephora* and "*Congensis* hybrid." *Robusta* and *quillou* were not different from the controls. Further inoculations into young seedlings of *robusta* (from local planta-

<sup>1</sup> Plants grown from seeds supplied by the Mayaguez Experiment Station, Porto Rico.

tion seed) resulted in the ringing and death of one plant out of four. It appears therefore that, while species other than *arabica* are generally resistant to this disease, occasional susceptible individuals may occur in the mixed population which is usually embraced by the term "*robusta*."

#### CONTROL.

In general the most successful method for combating diseases caused by fungi of the genus *Fusarium* is by the use of resistant varieties of plants. But this method is difficult of application in any long-period plantation crop. Still less application has it to my problem, which is the control of a disease in plantations now already existing. It is therefore hardly appropriate to point to the possibility that from the mixed *arabica* of this district resistant strains might be bred, or to point to the apparently high resistance of *robusta* types. These considerations would only be pertinent if the disease assumed much greater importance than at present, and if large new developments of coffee planting were in progress in the district where it occurs.

On the other hand, there is little prospect of successful control by direct methods. An attempt was made to reduce secondary spread of the fungus through the stumps, by excision down to the wood of all discoloured tissue around the base of dead shoots. This necessitated the removal of large areas of bark in many instances. The exposed area was painted with an antiseptic solution. The experiment demonstrated the possibility of a permanent excision of the fungus, since usually the margins of the excised areas developed a healthy callus all round. But this procedure failed to control the disease, owing probably in part to new primary attacks and in part to spread of the fungus within the stump tissues from overlooked centres of infection, where shoots had in the past died and fallen off. Even if this procedure were successful, however, it is considered to be an impracticable one for adoption as a field operation.

#### SUMMARY.

A disease of the bark of young leader shoots of *arabica* coffee occurs in the Usambara Mountains of Tanganyika Territory. The disease may be important when "stumping" is practised in the regeneration of old plantations. A brown sunken lesion, formed of dead extra-cambial tissues, usually gradually extends and rings the shoots; after a period varying from a few days to several months after complete ringing the foliage above wilts and dies. A leaf spot is also due to the same cause.

The fungus, *Fusarium lateritium* Nees var. *longum* Wr., among several isolated from lesions, has been shown to be capable of reproducing the disease by pure culture inoculation. A proportion of the experimental inoculations resulted in the ringing and death of the shoots; many of the lesions, however, after a time ceased to advance and became callused at the margin. Similar recoveries from the disease were observed in the field.

Field observations and experiments demonstrated that a common mode of entry of the fungus into the stem tissues was through freshly exposed leaf scars, and occasionally from a leaf-spot down the petiole of a leaf. Many shoots also in the field became diseased as the result of the fungus passing to them through the tissues of a stump from the base of a dead shoot.

*Coffea arabica* alone, of a number of coffee species tested, was susceptible to any extent to this disease.

Excision of the affected bark of a stump was effective in checking the spread of the fungus through the stump. The operation, however, is not considered to offer a practicable means of control in the plantation.

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## EXPLANATION OF PLATES VIII, IX.

## PLATE VIII.

- Fig. 1. Typical constriction produced by this disease in an old shoot from a stump in the plantation. The leaves of this shoot had recently wilted at the time that the photograph was taken.
- Fig. 2. Extreme form of constriction in stem of an inoculated plant. The inoculation was made with tissue from a natural lesion. The stem was ringed in about 9 months. Photographed 20 months after inoculation, at which time foliage above the "waist" was yellow but unwilted. This plant was still developing new leaves and was unwilted 7 months later. The lesion at this time was still spreading down the stem and into the side branches but had been checked by callus growth above.
- Fig. 3. Healing lesion on inoculated plant. The inoculation was made with tissue from a natural lesion. Photographed 23 months later, when callus was actively growing from the margins of the lesion.
- Fig. 4. Lesion produced on stem of young seedling by pure culture inoculation of *Fusarium lateritium* on an unwounded fresh leaf scar. The fungus had invaded both interpetiolar stipules and passed round the node and down the internode. Photographed two months after inoculation, at which time the top was beginning to wilt. The buds at the node below had meanwhile grown out into new leader shoots. (The black spherical objects above one stipule are insects.)

## PLATE IX.

- Fig. 5. The last stage of the disease. Stumped *arabica* coffee-plant in plantation, showing one shoot recently wilted, two shoots dead and leafless, and the stubs of two other shoots which have died and broken off.
- Fig. 6. Natural lesions on young green shoot from a stump in the plantation.
- Fig. 7. Leaf-spots produced by inoculation of a pure culture of *Fusarium lateritium* into wounds in a leaf of a coffee seedling. The opposite leaf was wounded and inoculated with sterile agar. Photographed 3 weeks after inoculation.
- Fig. 8. Lesions produced in young shoots from a stump by inoculation by hypodermic needle of a spore emulsion of *F. lateritium*.

(Received July 3rd, 1931.)



Fig. 3



Fig. 2



Fig. 1



Fig. 4







Fig. 5.



Fig. 6.

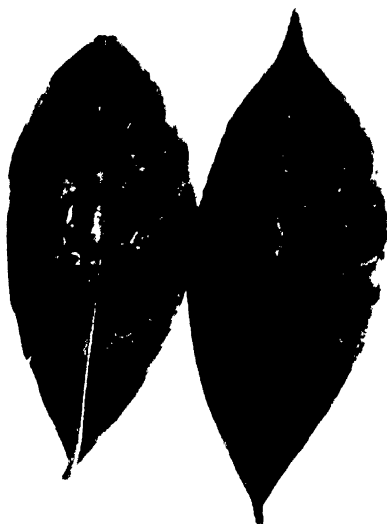


Fig. 7.

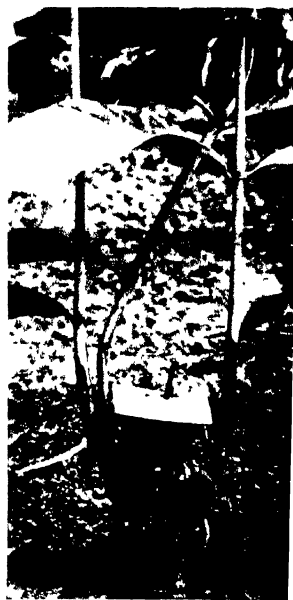


Fig. 8.



## THE CONTROL OF TOMATO-LEAF MOULD

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THE control of tomato-leaf mould caused by *Cladosporium fulvum* is a matter of great importance to growers of this crop, for while nurseries do exist in which infection is slight, there are hundreds of localities where the disease has a devastating effect.

Most workers on the subject have been impressed by the effect of atmospheric temperature and humidity on the incidence of this disease, and recently Small<sup>(2)</sup> showed that under the conditions appertaining in the glasshouses of this country leaf mould developed very rapidly at 20 and 22° C. and very slowly at 15° C. He also showed that humidities above 90 per cent. are extremely favourable and that at the optimum temperature of 22° C. infection is severe when the atmospheric humidity is 80 per cent. but rare at 70 per cent. As a rough guide, growers should keep the temperature of the houses below 21° C. (70° F.) and the humidity below 70 per cent. if the disease is to be kept in check.

Forced ventilation by means of fans or similar devices has been tried and would no doubt be of assistance, but in the absence of a cheap method the grower can only attempt to provide the right conditions by keeping up the night temperature and providing maximum ventilation whenever possible.

The intensity of the disease in August and September depends very largely upon the date of its first appearance. Thus in seasons when leaf mould appears in May it usually reaches serious proportions in late July, but if the initial symptoms are delayed until June the intensity in August is not so marked. Growers can do much to delay the appearance of the disease by starting to ventilate early in the season.

Fluctuation in temperature is also important, and some evidence was obtained during 1931 to suggest that where the night temperatures are maintained at a reasonably constant level, as is the case when completely automatic oil firing is employed, the incidence of the disease and its intensity are reduced. Thus it is obvious that while growers in some localities can reduce the extent of the disease by careful attention to house management, it would seem that they cannot always hold it in satisfactory control by this means.

The use of resistant varieties would offer the easiest means of overcoming the difficulty. Certain varieties such as "Up-to-date" and "Sterling Castle" are remarkably resistant, but unfortunately the quality and quantity of the crop are not satisfactory. There is reason to believe that varieties combining crop excellence with resistance will be obtained ultimately, but the work is necessarily slow, and it will be some time before they are available.

In the meantime the practice of control by spraying has been greatly improved. Originally the chief difficulty centred round the question of wetting the spore masses or pustules, which form on the under-surface of the leaves. These resisted successfully the action of the standard wetting agents such as soap, saponin, calcium caseinate, etc. Recently the application of certain sulphonated compounds<sup>1</sup>, which had been used previously in scouring wool and wetting cotton fibres, proved successful, and it was shown that water treated with such a compound at the rate of 1 oz. to 4 gallons was capable of wetting very thoroughly the pustules of *Cladosporium fulvum* on tomato foliage, without causing any injury to the growing plant. Once this was established it merely became a question of selecting a suitable fungicide for use with it.

Small<sup>(3)</sup> tested a number of fungicides compounded with Agral I and saponin respectively against *C. fulvum*, and obtained best results with ammonium copper carbonate, a colloidal sulphur preparation and the sodium salt of salicylanilide. The sodium salt of salicylanilide was first recommended by Galloway, Fargher and Probert, of the Shirley Institute, for preventing the development of mould in cotton goods<sup>(1)</sup>, and our attention was first drawn to it by Dr W. B. Brierley, of Rothamsted Experimental Station, who had found it highly toxic to certain fungi under laboratory conditions<sup>2</sup>.

Small tested ammonium copper carbonate with Agral I on tomato nurseries in the Lea Valley towards the end of 1930, and obtained good results without any scorching of the foliage. This mixture was tried again in the early part of 1931, with definite scorch on most nurseries and severe damage in a few houses. It is probable that the absence of any damage during the previous year was due to the condition of the foliage. This view is confirmed by the fact that the spray has been used in other parts of England during 1931 without injuring the plants. The

<sup>1</sup> *E.g.* one sold by Imperial Chemical Industries, Ltd., under the name of Agral I, which was the one used in the present experiments.

<sup>2</sup> This substance has been put on the market under the name Shirilan NA.

fact that scorching was produced under some conditions made it necessary to devise another mixture.

Colloidal sulphur with Agral I was found to check attacks of leaf mould to some extent, but the fungus grew out of the leaf again fairly quickly, and the relatively heavy deposit of sulphur on the fruit necessitated wiping it before marketing. This is a distinct disadvantage in commercial work.

Table I.

*Experiments in which the killing effect of certain compounds used in conjunction with Agral I was tested on spore cultures and on infected plants.*

Date 1931)	Fungicide (percentage concentration)				Material used	Effect on fungus		Effect on plant
						Pustules	Spores	
une 8	Ammonium carbonate	copper 0.45	Agral I 0.15		Tomato 5 months	*Dried up	—	Slight scorch
" 11	Colloidal sulphur	0.45	" 0.15		"	"	—	No scorch
" 17	"	0.45	" 0.15	Spore culture	"	—	Killed	—
" 17	"	0.15	" 0.15	"	"	—	None killed	—
" 26	"	0.45	" 0.15	Tomato (30 plants)	Dried up	—	—	Heavy deposit sulphur on foliage and fruit
uly 3	"	0.45	" 0.15	Tomato (45 plants)†	"	—	—	"
" 3	"	0.15	" 0.15	Spore culture	"	—	None killed	—
" 3	"	0.3	" 0.15	"	—	—	Some killed	—
" 3	"	0.45	" 0.15	"	—	—	Killed	—
" 7	"	0.3	" 0.15	"	—	—	Some killed	—
" 7	"	0.45	" 0.15	"	—	—	Killed	—
" 3	Colloidal copper	0.45	" 0.15	"	—	—	None killed	—
" 7	"	0.45	" 0.15	"	—	—	"	—
" 7	"	0.3	" 0.15	"	—	—	"	—
" 7	"	0.6	" 0.15	"	—	—	Majority killed	—
" 7	"	0.6	" 0.15	Tomato (6 plants)	Partially dried up	"	—	Scorched young growth. Slight stain on foliage and fruit
une 17	Salicylanilide paste	0.15	" 0.15	Spore culture	—	—	Killed	—
" 17	"	0.3	" 0.15	Tomato plants	Dried up	—	—	No damage
" 24	"	0.15	" 0.15	Spore culture	—	—	Killed	—
" 26	"	0.15	" 0.15	Tomato (30 plants)	Dried up	—	—	No damage
uly 3	"	0.15	" 0.15	Tomato (45 plants)†	"	—	—	"
" 7	"	0.075	" 0.15	Spore culture	"	—	Killed	—
" 7	"	0.04	" 0.15	"	—	—	Some killed	—
" 16	"	0.075	" 0.15	"	—	—	Killed	—
" 16	"	0.04	" 0.15	"	—	—	Some killed	—
" 18	"	0.075	" 0.15	Tomato (30 plants)	Dried up	—	—	No damage

\* When the pustules are "dried up" the spores are killed. † Thirty of these plants were sprayed 1 week previous.

The sodium salt of salicylanilide was also tried with Agral I and, while pustules of the fungus were destroyed, serious scorching of the foliage resulted. Ultimately salicylanilide itself dispersed in water as a 50 per cent. paste<sup>1</sup> was tried in conjunction with Agral I. This proved effective in destroying the pustules without injuring the foliage.

<sup>1</sup> This has been put on the market by Imperial Chemical Industries, Ltd., as Shirilan Paste.

It will be seen from Table I that  $\frac{1}{8}$  oz. salicylanilide paste in 1 gallon of water (0.078 per cent.) was sufficiently strong to control tomato-leaf mould, and experiments were arranged to determine the number of applications required to stop the disease once it had become well established in a glasshouse. From August 19th onwards badly infected plants were sprayed at weekly and fortnightly intervals, and it was found that where a second spraying had been given a week after the first application, the disease did not develop round the edge of the old pustule for another 3 weeks, but where the second spraying was delayed for a fortnight, the edge of the old pustule had started to develop before the second spraying had been given. This has been confirmed fully by trial on commercial nurseries.

It is therefore possible to recommend the following mixture as a spray for controlling tomato-leaf mould:

$\frac{1}{8}$  oz. salicylanilide paste.  
 $\frac{1}{4}$  oz. Agral I.  
1 gallon of water.

The powder, Agral I, should be sprinkled into the water and mixed by stirring vigorously. Afterwards the requisite amount of salicylanilide paste may be added and also mixed by stirring. The spray should be applied at the onset of the disease, for when this is done a second spraying 7 days later is sufficient to check its progress for a considerable time. If spraying is left until infection is severe the difficulty of controlling it is intensified greatly.

In preliminary experiments the above spray mixture has also provided a satisfactory control of cucumber mildew—*Erysiphe cichoracearum*—although it has not been possible to experiment with very young plants on a large scale, and more work is necessary.

Also powdery mildew of the chrysanthemum (*Oidium Chrysanthemi*) and of the rose (*Sphaerotheca pannosa*) has been controlled after a few applications, and it is possible that the spray may prove as useful against other diseases of glasshouse plants.

#### SUMMARY.

1. Tomato-leaf mould may be held in check by attention to atmospheric temperature and humidity.

2. Varieties resistant to this disease are known, but they are not suitable for commercial work. Attempts are being made to breed varieties combining resistance with crop excellence.

3. The disease has been controlled by spraying with salicylanilide paste, sold under the name of "Shirlan Paste," combined with a sulphonated oil sold as Agral I.

4. The above spray mixture has also proved effective against certain other diseases.

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(Received December 17th, 1931.)



## STUDIES IN BACTERIOSIS

## XIX. RESEARCHES ON THE GROUP OF GREEN-FLUORESCENT BACTERIA, PART II; ON SOME PLANT DISEASES CAUSED BY BACTERIA OF THE GREEN-FLUORESCENT GROUP, AND A COMPARISON AND DISCUSSION OF VARIOUS CULTURAL CHARACTERISTICS OF CERTAIN MEMBERS OF THIS GROUP

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In Part I of this series it was stated that pathogenic strains of bacteria of the green-fluorescent group had been isolated from diseased broad beans, potato tubers, lettuces, and *Medicago lupulina* seeds, and a description of the first-named disease, with an account of the identification of the causal organism as *B. trifoliorum*, was given. The present paper describes the strains isolated from the other three host plants and compares the cultural characters of these, and certain strains isolated from other plant lesions, with known bacterial species.

## A. RING DISEASE OF POTATO TUBERS; CAUSAL ORGANISM STRAIN 221.

In May, 1929, potato tubers were examined which had extensive dark brown, corky lesions, with an occasional cavity, in the vascular ring. In one tuber the pith was necrosed and light brown in colour in addition to the lesions in the vascular ring, but externally all the tubers appeared sound. The lesions suggested an attack by *B. solanacearum* or *B. sepedonicum* and these organisms were looked for, but without success. On the other hand, however, an organism (strain 221) was obtained which, when inoculated on to potato slices, produced a typical rot of the vascular ring accompanied by strong browning, and in some cases a slight necrosis of the pith. In one experiment potato slices were inoculated and incubated at 20, 25 and 30° C. respectively; at all these temperatures considerable rot of the vascular ring at the points of inoculation ensued, being most extensive at 30° C., at which temperature the necrosis had spread throughout the vascular ring in 5 days. Whole tubers, inoculated by a needle prick and held at room temperature, in

one experiment developed typical lesions, a dark brown necrosis developing round the inoculation prick and extending for a considerable distance through the vascular ring. In another test, when the potatoes were kept under moister conditions, a general rot of the tissue around the pricks developed; this rot differed from a soft rot induced by *B. phytophthorus* or *B. caratovorous*, in that the middle lamella was not dissolved.

*Description of strain 221.*

A short rod, motile by means of 1-5 polar flagella, aerobic, gram-negative, no spores, optimum temperature 30° C., no growth at 37° C. No production of indol; diastatic action very feeble; gelatine liquefied; nitrates reduced to nitrites with slight evolution of gas. Acid but no gas from glucose, galactose and feebly from saccharose; none from lactose, mannite, maltose, dulcitol, sorbitol, salicin or glycerine. Milk becomes strongly alkaline, digestion occurs without coagulation, litmus is reduced. Good growth in Fermi's and Uschinsky's solutions with yellow-green fluorescence. Green colour is also produced in gelatine media and sometimes in bouillon. Colonies on bouillon agar plates pH 6.8-7.0 have raised opaque yellowish centres with flat white translucent borders, frequently exhibiting internal concentric markings and striations, margins entire or slightly crenated; the agar becomes strongly browned. In one sub-culture from a strongly fluorescent gelatine plate to a bouillon-agar slope, the medium after 24 hours had a green tinge but later the usual brown colour developed. In cultures on pea-extract agar the medium became pink.

*Comparison of strain 221 with known bacterial species.*

*Strain 221* is very closely related to *B. xanthochlorum* (Schuster), with which it agrees not only in most of its cultural characteristics but in pathogenicity. Both cause a vascular rot of potato tubers, but are non-pathogenic to potato stems and also to tomato plants; and both produce a vigorous rot of broad beans. Brown and Jamieson<sup>(3)</sup> compared *B. xanthochlorum* with *B. aptatum* (isolated by them from sugar beet and nasturtium-leaf lesions) and concluded that the marked green fluorescence in nearly all media and the formation of alkali instead of slight acid in saccharose broth in *B. xanthochlorum* cultures were the only real points of difference between the two. In both these characters strain 221 agrees with *B. aptatum*. Brown and Jamieson also compared *B. aptatum* with *B. tenuis* (isolated from maple sap by Edson, Jones and Carpenter<sup>(8)</sup>), and found the difference between them to be physio-

logical rather than cultural, for *B. tenuis* was non-pathogenic to nasturtium and sugar beet. Of the saprophytic bacteria, *B. fluorescens liquefaciens* most nearly approaches strain 221, and Israelsky and Runov<sup>(13)</sup> found that a close relationship existed between *B. fluorescens liquefaciens*, *B. xanthochlorum* and a strain "B. No. 1" isolated by them from a diseased potato tuber.

#### B. DISEASE OF LETTUCES.

Numerous isolations from various types of lesions on lettuce leaves have been made, and in every case the causal organisms have proved to be of one type, very similar to the strain described by Paine and Branfoot<sup>(10)</sup> as causing a disease of lettuces in England in 1921. The strains which were used in most of the cultural and inoculation work are described below.

(1) In June, 1929, isolation plates were made from lettuces which had a marginal rot of the leaves. A virulent organism was isolated which on inoculation not only caused a marginal rot identical with the original disease but also attacked the midribs of the leaves.

##### *Description of the lettuce organism, strain 224.*

A short rod, motile by means of 1-4 polar flagella, aerobic, no spores, optimum temperature 30° C., no growth at 37° C. Gram stain doubtful (when stained on the same slide as true gram-negative and gram-positive organisms it was intermediate between them). No production of indol; no diastatic action; no reduction of nitrate; gelatine liquefied. Acid but no gas produced from dextrose, galactose and feebly from saccharose; none from lactose, mannite, maltose, dulcitol, sorbitol or salicin. Milk becomes strongly alkaline and digestion occurs without coagulation, no reduction of litmus. Good growth in Fermi's and Uschinsky's solutions with formation of slight yellow-green fluorescence. No pigment was produced in gelatine or agar media or in broth. Growth on bouillon agar yellowish white; colonies on the same plate varied from round, translucent, entire margin, with raised centres and flat, more transparent borders, to a flat spreading type very similar to those of strain 212 (isolated from broad beans) but less transparent.

(2) In August, 1928, a strain (203) had been isolated from a soft rot of young heart leaves of lettuce and this strain was found to be still virulent in June, 1929. Strain 203 agreed very closely with strain 224, the only points of difference being that strain 203 produced a soft clot

in milk, and very strong green fluorescence in nearly all media, including gelatine, nutrient agar and bouillon.

(3) In November, 1929, lettuces were obtained which had yellowish brown necrotic lesions on the midribs of the inner leaves, the outer leaves being dead. The lesions were similar in appearance to those produced by inoculation of *strain* 224, and an organism (233) was isolated from them which was culturally identical with that strain except that it was definitely gram-negative, produced a slight green fluorescence in beef agar and bouillon, and reduced litmus.

(4) In August, 1930, *strain* 248 was isolated from a marginal rot of lettuce. This agreed culturally with *strain* 233.

(5) *Strain* 238 was isolated in March, 1930, from a large rotted lesion on a young heart leaf of lettuce. Inoculation with this strain produced an extensive marginal rot and also a rot of the mid-ribs of lettuce leaves. Culturally *strain* 238 agreed with *strain* 203, except for a feeble power of reducing nitrate to nitrite and feeble growth at 37° C.

#### *Comparison of the lettuce strains with known bacterial species.*

Nellie Brown<sup>(1)</sup> described three bacterial species producing disease in lettuce—*B. marginale*, *B. vitians* and *B. viridilividum*. Of these *B. marginale* (later identified by Mehta and Berridge<sup>(17)</sup> as a strain of *B. pyocyaneus*) is the most closely related to the five strains 203, 224, 233, 248 and 238, but differs from the first four in that it reduces nitrate and grows at 37° C. The fifth strain, 238, is an intermediate form in both these characters.

Another closely allied species is *B. aptatum* (referred to above with reference to the potato disease), and Brown and Jamieson<sup>(3)</sup> obtained diseased spots on lettuces by inoculation with this organism. In a recent paper Burkholder<sup>(7)</sup> described six bacterial species causing disease of *Phaseolus vulgaris*, of which three (*B. medicaginis* var. *phaseolicola*, *B. viridiflava* and *B. vignae* var. *leguminophila*) belong to the green-fluorescent group. Of these the latter strain appears very similar to the lettuce strains described above both in cultural characteristics and in pathogenicity on various hosts. This species is the only one of Burkholder's cultures which attacked *Vicia faba*, on which it produced lesions similar to those obtained by inoculation with the lettuce strains.

C. DISEASE OF *MEDICAGO LUPULINA* SEEDS.

## CAUSAL ORGANISM STRAIN 234.

In December, 1929, seeds of *Medicago lupulina*, with large brown necrotic lesions on the testa, were examined. Some of the seeds were planted but only 33 per cent. germinated and of these several withered, while others bore large brown lesions on the cotyledons. Identical bacterial strains were isolated from the lesions on the cotyledons and from the original spots on the seeds, and these strains were found to be virulent for broad beans, lettuce and potato tubers.

*Description of strain 234.*

A very short rod, motile by one polar flagellum, gram-negative, aerobic, no spores, optimum temperature 30° C., no growth at 37° C. Very feeble indol production and diastatic action; gelatine liquefied, no reduction of nitrate. Acid but no gas from dextrose, galactose and very feebly from saccharose; none from lactose or glycerine. Milk becomes slightly alkaline, with a soft coagulum which is digested. Slow production of yellow-green fluorescence in Fermi's and Uschinsky's solutions, and also in gelatine media. Colonies on bouillon-agar plates are variable, all stages from round raised colonies with entire margins to a flat spreading type appear on the same plates. A slight fluorescence is sometimes developed in the agar, and there is a copious development of crystals in the medium beneath the colonies, giving the growth a chalk-white appearance.

*Comparison of strain 234 with known bacterial species.*

The six species *B. pisi*, *B. aptatum*, *B. delphinii*, *B. syringae*, *B. coronafaciens* and *B. papulans* are all very similar culturally to strain 234. Of these *B. pisi* is perhaps the most closely related, but Sackett<sup>(20)</sup> finds this species to be non-pathogenic to *Vicia faba* while strain 234 is strongly virulent on this host. It is possible that strain 234 is a physiological variety of *B. pisi*.

*Comparison and discussion of cultural characters of members of the green-fluorescent group.*

In addition to the strains already described in some detail above (221 from potato; 203, 224, 233, 238 and 248 from lettuce; 234 from *M. lupulina*) and the strains from broad beans (212) and clover (202) described in the first paper of this series, the following strains are included in this comparison: (a) strain 245 isolated from diseased mush-

rooms in June, 1930, identified as *B. tolaasi*; (b) several strains isolated from Larkspur lesions identified as *B. delphinii*; (c) several strains, agreeing with *B. papulans*, isolated from blister-bark disease of apple trees; and (d) various unidentified strains from lesions on certain other plants. Cultures of *B. delphinii*, *B. marginale* and *B. aptatum* (obtained from America through the National Collection of Type Cultures) and the saprophytic species *B. fluorescens-liquefaciens* and *non-liquefaciens* and *B. pyocyaneus*, have also been used in cultural comparisons with the above strains.

Burkholder<sup>(6)</sup> has given tables of the cultural reactions of the *Phytonomonas* group of bacteria, including members of the green-fluorescent group. It was stated by the writer in the first paper of this series that several species of Burkholder's "colourless" forms produce green-fluorescent pigment on certain media, and these have been included in the present work as belonging to the group under discussion. It is probable that there are still other species which should be included, for "green fluorescence" is a very variable and uncertain character. To give examples: (a) Smith<sup>(21)</sup> states that from a culture of *B. xanthochlorum* obtained from Schuster, Clara Jamieson isolated a non-fluorescent strain which retained this character in all media studied; in other respects this strain resembled the parent. (b) L. R. Jones<sup>(15)</sup> isolated two strains of *B. trifoliorum*, both pathogenic to clover, and similar in most respects, but one strain was fluorescent on beef media, etc., while the other gave a slight green colour in Uschinsky's medium only. (c) Nellie Brown<sup>(2)</sup> isolated an organism from geranium leaf-spot which was very similar to *B. erodii* (Lewis) but which never produced any colour at any time. She says, "Could this pigment formation be the individuality of a strain? If so it is a striking variation and a feature that must be reckoned with when comparing strains and varieties in proving up a new organism." (d) The same variations occur with the production of pyocyanin, and Hadley<sup>(11)</sup> states that a pyocyaneus culture may gradually lose its power to produce blue pyocyanin and become a "fluorescengenetic but non-pyocyanogenic organism." (e) In the present work, of two strains isolated from lettuce, strain 203 produced a very strong fluorescence in all media while strain 224, which otherwise closely resembled strain 203, produced no pigment in any media, except a feeble green colour in Uschinsky's medium.

*The influence of temperature and hydrogen-ion concentration on the production of fluorescent pigment.*

While tests on optimum temperature and hydrogen-ion concentration were being made with certain of the strains it was noticed that there was considerable variation in the amount of fluorescence produced in the broth cultures under different conditions. These tests were therefore repeated with nine of the pathogenic strains and also with cultures of the saprophytic species *B. fluorescens liquefaciens* and *B. fluorescens non-liquefaciens*. In each case 5 c.c. of bouillon was inoculated with one loopful of a 24-hour culture of the organism. In the series in which the temperature was the varying factor the initial pH value of the broth was 7.2, and the tubes were incubated at room temperature (about 16° C.), 20, 22, 25 and 30° C. respectively. In the varying hydrogen-ion concentration series the pH value of the broth was 5.2, 5.8, 6.9, 7.2 and 7.6 and 8.2 respectively, and the tubes were incubated at 25° C. (The pH values were estimated colorimetrically after sterilisation.) The tubes were examined for growth and fluorescence over a period of 3 weeks. In every case the optimum temperature for growth was between 25 and 30° C. There was very little difference in the growth of any of the strains in the broths ranging from pH 5.8 to 6.9, but less growth developed in broths of pH 7.2, 7.6 and 8.2 except in the cultures of *B. fluorescens non-liquefaciens* which grew equally well in all tubes from pH 5.8 to 8.2. No growth occurred after 24 hours' incubation in any of the pH 5.2 broth tubes; on the second day there was good growth with *B. fluorescens liquefaciens* and *non-liquefaciens*; on the third day growth occurred in tubes inoculated with four of the parasitic strains (234, 238, 248 and 245) and on the sixth day with strain 221. pH 5.2 tubes inoculated with strains 212, 202, 203 and 224 remained sterile. Tables I and II show the development of fluorescence in these cultures.

It appeared from the results shown in Table II that the hydrogen-ion concentration was a very important factor in the production of fluorescence, and as strain 203 had produced this pigment in broths from pH 5.8-6.9 on the sixth day while *B. fluorescens liquefaciens* and *B. fluorescens non-liquefaciens* had failed to do so, a possible explanation was that strain 203 had formed a greater amount of alkali than the other two and so had rendered the medium favourable for fluorescein development. The pH values of these cultures and of some of those used in the experiment of Table I were therefore tested, and it was found that this explanation would not entirely fit the facts. Thus after 6 days

the *pH* of broth whose initial value was 5.8, 6.6 or 6.9 had become 7.4, 7.8 and 8.4 respectively in the tubes inoculated with *strain* 203, in which fluorescein had developed, and 7.6 and 8.4 in the tubes inoculated with *B. fluorescens liquefaciens* and *B. fluorescens non-liquefaciens*, in which there was no fluorescence. Also the *B. fluorescens non-liquefaciens* broth culture incubated at 16° C. showed slight fluorescence, the other two none, but all these were of equal *pH* value. A further interesting fact was observed; when tubes showing fluorescence were acidified to about *pH* 6.2 the green colour immediately disappeared, but returned on adding weak alkali to the broth. On the other hand, when alkali was added to the cultures in which no fluorescence was observable, the pigmentation promptly developed in the tubes of the temperature series, but despite the fact that there was very vigorous growth no colour at all was observable in the *B. fluorescens liquefaciens* and *non-liquefaciens* cultures of the *pH* series.

Table I.

*Effect of temperature on fluorescence.*

Strain	Host plant	Temperature. ° C.				
		Room	20	22	25	30
<i>B. fluor. liq.</i>	—	- ⊙	- ⊙	±	+	++
<i>B. fluor. non-liq.</i>	—	+	+	+	++	++
212	Broad bean	-	-	-	+	+
202	Clover	-	-	-	+	+
221	Potato	±	±	±	+	+
203	Lettuce	⊙	+	+	++	++
224	"	-	-	-	-	- (Brown)
238	"	±	±	±	+	+
248	"	-	-	-	-	+
234	Medicago	±	±	±	⊙	+
245	Mushroom	±	±	±	+	+

- no fluorescence; ± slight fluorescence on fifteenth day; + fair; ++ very strong fluorescence; ⊙ used for tests on the sixth day, no fluorescence.

It is obvious that, except in the case of very vigorously fluorescing strains such as 203, differences in the medium and in the temperature exert a great influence on fluorescein formation, and this character cannot be altogether relied on in the differentiation of species. Thus Tables I and II show that, of the lettuce strains, 203 and 238 produced fluorescence in media over a wide range of *pH* values and temperatures, *strain* 248 only in broth of *pH* 8.2, or at a temperature of 30° C. in *pH* 7.2 broth, while 224 produced no fluorescein under any conditions. Also, when identifying a newly isolated strain, the power of fluorescein production may be overlooked through the use of unsuitable media or



temperature. Thus, bouillon agar of about pH 6.8 has been found the most satisfactory for general use, but on this medium several of these strains will not produce fluorescence and the same negative result is obtained even on more alkaline media at a temperature of 22° C. or lower.

Table II.

*Effect of hydrogen-ion concentration on fluorescence.*

Strain	Host plant	pH of broth						
		5.2	5.8	6.6	6.9	7.2	7.6	8.2
<i>B. fluor.-liq.</i>	—	—	—	—	—	++	++	++
<i>B. fluor. non-liq.</i>	—	—	—	—	—	++	++	++
212	Broad bean	—	—	—	—	+	+	+
202	Clover	—	—	—	—	+	+	+
221	Potato	—	+	+	+	+	+	++
203	Lettuce	++	++	++	++	++	++	++
224	"	—	—	—	—	—	—	—
238	"	±	+	+	+	+	++	++
248	"	—	—	—	—	—	+	+
234	Medicago	—	—	—	—	⊙	+	+
245	Mushroom	—	—	—	—	+	+	++

Signs as for Table I.

*Physiological reactions of the green-fluorescent group.*

Burkholder<sup>(6)</sup> gives tables of the cultural reactions of the *Phytomonas* group of bacteria and the percentages of like characters found in various groups. Following this method, Table III gives both the actual number and the percentages of some of the characteristics of thirty-six strains of green-fluorescent plant pathogens isolated during the present investigation, while Table IV is a similar list of thirty-two species of, or closely related to, this group, compiled partly from Burkholder's figures but including some species not given by him. In these tables aerobism has been omitted, because, while all the strains and species are markedly aerobic, some are stated to be facultative anaerobes owing to their occasional scanty growth under anaerobic conditions, *e.g.* *B. medicaginis* var. *phaseolicola*, which sometimes, but not always, grows in the closed arm of fermentation tubes containing glucose and saccharose broth.

An explanation of the use made here of the word "strain" and the number of strains comprising the thirty-six given in Table III is perhaps desirable. In most cases one "strain" represents isolations from one lot of diseased material, when all the virulent colonies were alike. For example, from isolations from the broad-bean disease (causal organism 212) a number of colonies were tested and all found to be culturally identical; also all the re-isolations from various plant inoculations agreed

with the original cultures. All these cultures were therefore placed together in one strain, 212. Occasionally, however, cultures made from separate colonies from a single isolation plate would differ slightly in some particular character; for example, of two colonies from black-spot of larkspur one produced acid from sucrose, the other did not; and of similar isolations from blister-bark of apple, in one case there was a difference in indol production, and in another in the peptonisation of milk. Such varying colonies have been called different strains. Except for these cases, several strains from one host plant, as in apple and lettuce, represent isolations made at different times from different lots of material.

Table III.

*Distribution of characters in thirty-six strains of green-fluorescent plant pathogens.*

Character	Total number				Percentages	
	Not given	Positive	Strains and host plant	Negative	Strains and host plant	Positive Negative
Gram stain	—	? 1	Doubtful positive, 1 lettuce	35	—	3 97
Milk; alkaline	4	28	11 apple, 4 delphinium, 6 lettuce, 1 clover, 1 bean, 1 pea, 1 potato, 1 medicago, 1 parsnip, 1 cauliflower	4 (No change)	1 apple, 1 lettuce, 1 mushroom, 1 potato	87.5 12.5
Milk; curd	4	9	1 apple, 1 delphinium, 4 lettuce, 1 medicago, 1 mushroom, 1 cauliflower	23	11 apple, 3 delphinium, 3 lettuce, 1 clover, 1 bean, 1 pea, 2 potato, 1 parsnip	28 72
Milk; peptonised	4	29	11 apple, 4 delphinium, 7 lettuce, 1 medicago, 1 mushroom, 2 potato, 1 pea, 1 cauliflower, 1 parsnip	3	1 clover, 1 bean, 1 apple	91 9
Liquefaction of gelatine	—	34	All strains except clover and bean	2	1 clover, 1 bean	94.5 5.5
Acid from dextrose	—	36	All strains	0	—	100 0
Acid from lactose	—	0	—	36	All strains	0 100
Acid from sucrose	—	26	12 apple, 4 delphinium, 6 lettuce, 1 clover, 1 bean, 1 medicago, 1 potato	10	2 apple, 1 lettuce, 1 mushroom, 1 potato, 2 delphinium, 1 pea, 1 cauliflower, 1 parsnip	72 28
Reduction of nitrate	12	3	1 lettuce, 1 potato, 1 cauliflower	21	6 apple, 4 delphinium, 6 lettuce, 1 clover, 1 bean, 1 mushroom, 1 medicago, 1 potato	12.5 87.5
Starch digested	19	12 weak	3 apple, 2 delphinium, 3 lettuce, 2 potato, 1 mushroom, 1 medicago	5	1 clover, 1 bean, 2 lettuce, 1 cauliflower	70.5 29.5
Indol	17	7 weak	1 apple, 3 lettuce, 1 clover, 1 mushroom, 1 medicago	12	2 apple, 2 delphinium, 4 lettuce, 2 potato, 1 bean, 1 cauliflower	37 63

It is difficult to determine to what extent, and in what combinations, the variations in these characters should be considered of specific value. The tables show that eight of the eleven characters given are in great predominance throughout the group. These are non-straining by Gram's

method; production of alkali in, and digestion of milk; production of acid from glucose and saccharose but not from lactose; liquefaction of gelatine and non-reduction of nitrate. Of the remaining three characters, about half the species in Table IV but only 28 per cent. of the strains in Table III produce a soft clot in milk. The other two characters, diastatic action and the production of indol, are never more than weakly positive, and it was found that varying results could be obtained by altering the temperature and time of incubation, and, in the case of indol formation, with different batches of media. Thus four strains (202, 212, 224 and 203) gave a negative result for diastatic action at 25° C. but a weak positive at 30° C., and two strains which produced no indol from one batch of broth gave a very little in another test. Also, strains otherwise identical may differ in the power to produce indol, as in the case of an isolation from blister-bark of apple.

Table IV.

*Distribution of characters in thirty-two species of the green-fluorescent group.*

Character	Total number			Percentages	
	Not given	Positive	Negative	Positive	Negative
Gram stain	—	1	31	3	97
Milk alkaline	4	26	2	93	7
„ curd	—	15	17	47	53
„ peptonised	—	28	4	87·5	12·5
Starch digested	5	11	16	41	59
Acid from glucose	1	28	3	90	10
„ lactose	—	0	32	0	100
„ saccharose	1	21	10	68	32
Liquefaction of gelatine	—	28	4	87·5	12·5
Reduction of nitrate	—	9	23	28	72
Indol	2	12	18	40	60

It has also been pointed out that the production of acid from saccharose (always very weak throughout the group) might differ even between two otherwise identical colonies taken from the same isolation plate. The fermentation of glucose is a far more constant character, being positive in all the strains tested in the present work, while only three of the species are negative. Burkholder<sup>(5)</sup> states that *B. medica-ginis* var. *phaseolicola* only produced very slight acid from both glucose and saccharose which is quickly masked by alkaline formation, and it seems possible that this may occur with other strong alkali producers, particularly in saccharose broth cultures, and that a feeble and transient acid reaction may be overlooked or entirely counteracted by the alkali produced from the peptone.

The fifth and sixth characters to be considered are the coagulation and peptonisation of milk. While the production of alkali in milk is an almost constant character throughout the group, the extent and rapidity of coagulation and digestion vary considerably. Jones<sup>(15)</sup>, for example, found that of two strains of *B. trifoliorum* isolated from leaf-spot of clover, although in each case there was a very slow production of a curd (5 weeks), one strain digested the casein, the other did not, and Elliott<sup>(9)</sup> found that *B. coronafaciens* and *B. striafaciens* usually formed a soft curd which was slowly peptonised, but sometimes the milk was cleared without formation of a clot. Among the strains investigated the same variations occurred. The majority of cultures isolated from blister-bark of apple peptonised but did not coagulate milk, but one failed to peptonise and a second formed a soft clot. Also, out of seven isolations from lettuce diseases, four of the strains coagulated milk and three did not, but all peptonised.

The last two characters to be considered, namely reduction of nitrate and liquefaction of gelatine, appear to be of more specific value than the rest, but even these cannot be relied on absolutely. *B. marginale* reduces nitrate, but of the seven lettuce strains mentioned above only one (238) gave a feeble positive result, thus being intermediate between *B. marginale* and the other strains, although there is a close agreement in all other respects. Other workers find the same variations. Thus Paine<sup>(18)</sup> isolated a strain from mushrooms which agreed with the organism of Tolaas<sup>(23)</sup> except that the former did not reduce nitrate. (In this character the recently isolated mushroom strain 245 agrees with Paine's culture.) Differing results may also be obtained by different methods of testing for nitrate. Elliott<sup>(9)</sup>, in her original paper on *B. coronafaciens*, states that it is a non-nitrate reducer: later, in a comparison of this species with *B. striafaciens*<sup>(10)</sup>, she found that both species gave a negative result by one method but a positive result by a more delicate method, and Bryan<sup>(4)</sup> obtained the same result with *B. syringae*.

Even the liquefaction of gelatine is not reliable, and discrepancies may occur owing to variations in the gelatine medium. Thus Burkholder, in his original description of *B. medicaginis* var. *phaseolicola*, states that no liquefaction occurs. Further work with the pathogene on other gelatines, however, showed that both the original strains and several later isolated strains produced a slight liquefaction after a fortnight, and at the end of 6 weeks the gelatine was like a heavy oil. (Recently, Hedges<sup>(12)</sup> found that *B. puerariae*, a gelatine liquefier, is a synonym of this species.) Several species only liquefy gelatine very slowly and

partially and *B. lacrymans* may be a non-liquefier or produce a very slow liquefaction. Jagger<sup>(14)</sup> records that various strains of *B. apii* show marked variation in the rate of liquefaction, and Schurmayer<sup>(22)</sup> observed, while working with *B. pyocyaneus*, that some descendants of an original liquefying culture had scarcely any power of liquefaction. During the present work it has been noted that certain strains became very slow liquefiers after being in culture for some time, although various attempts to complete this loss of proteolytic action, or to produce it in the case of non-liquefying strains, have not been successful.

Many other examples of variations could be given, but enough has been said to demonstrate the difficulties encountered in attempting to identify a newly isolated member of this group and to support Burkholder's<sup>(6)</sup> statement as to the confusion arising from the tendency to create new species on slight cultural differences.

#### SUMMARY.

Diseases of potato tubers, lettuces and seeds of *Medicago lupulina* caused by bacteria belonging to the green-fluorescent group are described and the identity of the pathogens is discussed. The cultural characters of thirty-six strains isolated from various plant lesions are given and compared with those of thirty-two known species of the green-fluorescent group, and a discussion on cultural variations follows.

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(Received July 20th, 1931.)

## AN ECOLOGICAL STUDY OF CLOSELY CUT TURF TREATED WITH AMMONIUM AND FERROUS SULPHATES

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(With 5 Text-figures.)

### INTRODUCTION.

INVESTIGATIONS on the influence of fertilisers on closely cut turf, such as is found on a lawn, were carried out at the Rhode Island Agricultural Experimental Station (6) as long ago as 1905-10. Following up these experiments, Hartwell and Damon (3) and Garner and Damon (2) showed that fertilisers brought about a wide range of vegetation changes and that those fertilisers with a "physiologically acid" soil reaction and high nitrogen content were the most active. In 1921 a Government-assisted Research Station was established to investigate problems of a biological nature in connection with golf courses. The influence of fertilisers on turf was one of the first problems to be investigated, and the results of the preliminary trials were published by Oakley (5) in 1925. He described a series of plots which had been sown down with *Agrostis tenuis* in 1921 and which received, during the summer and spring of 1922-5, periodic dressings of various fertilisers. Oakley concluded from these experiments that the key to the differential action of the various nitrogenous fertilisers lay in the degree of soil acidity established. Fertilisers with an acid reaction, such as ammonium sulphate, tended to retard weed growth and encourage the grasses, while sodium nitrate, on account of its alkaline reaction, reduced the acidity and thereby rendered conditions more favourable for the weeds.

As a result of these experiments, a general system of turf treatment was put forward for use on golf greens. The recommendations were that during spring and summer the turf should receive, at intervals of a fortnight to a month, dressings of ammonium sulphate at the rate of 3-5 lb./1000 ft.<sup>2</sup>, equivalent to 1.2-2.0 cwt./acre. The fertiliser was to be applied either as a dilute aqueous solution, or to be broadcast evenly

over the turf and immediately afterwards watered in with a hose to prevent "scorching." In addition, the turf was to receive from time to time dressings of compost made from leaf mould, sand and turf loam. On no account was the turf to be limed, care being taken that the sand of the compost did not contain calcium carbonate, as is usually the case with sea sand.

In 1927 it was decided to repeat some of the American work to discover whether the same system of manuring would be equally successful under English conditions. The first trials were carried out on the greens at Stoke Poges Golf Club near Slough, other centres being included later.

### *Experimental technique.*

*Botanical analyses.* Vegetation analysis of the floral composition of a lawn represents a special problem in that the extreme prostrate habit of the plants and constant cutting by the mower render impossible estimations either by tiller counts or by weights of the cut herbage. It was decided therefore that the most satisfactory method was to determine the percentage area covered by each species.

In the first investigations small fixed quadrats were used (4 sq. feet). Area estimations were made by means of a "grid," consisting of a wooden frame (1 ft.  $\times$  1 ft.) divided into a number of rectangles (2 in.  $\times$  1 in.) by means of a series of fine wires. By the subdivision of the surface into these small rectangles, the estimation of area covered became more accurate. A serious disadvantage inherent in the use of such small quadrats for observation came to light in the winter of 1927-8, in that any slight damage to the turf in the quadrat would be relatively so large as to render subsequent area counts unreliable. In one instance, owing to the mower being set too low, 25 per cent. of the area of the quadrat was removed by the blades.

On account of this danger of "scalping," the original technique was modified for all trials started subsequent to those of 1927. The area under observation was increased from the small quadrat to a square of 10 ft.  $\times$  10 ft. The grid was reduced in size to 6 in.  $\times$  6 in. and contained nine smaller squares (2 in.  $\times$  2 in.). The practice now adopted was to throw down the new small grid ten times at random on the plot and to estimate the areas covered by grasses and weeds at each throw.

To test the accuracy of this method of random sampling, a hundred grid estimations were made on the same plot during a period of 2 days. When the samples were summed in groups of ten, the resulting figures agreed within a small margin.



In the work of 1927, the vegetation was divided simply into grasses and weeds (*i.e.* species other than Gramineae), but in all subsequent determinations area estimations were made for each grass and weed species individually. All determinations under both methods were made immediately after the turf had been cut, thus reducing the risk of inaccuracies due to the obscuring of one species by another, such as would occur if the observation was deferred for a few days.

*Method of soil sampling for pH determinations.*

In order to study the changes in soil reaction brought about by the treatments, series of soil samples were taken initially and again after the application of the last dressing in each experiment. Determinations of *pH* were carried out by means of quinhydrone and gold electrodes. In the first experiments of 1927 these *pH* values showed that the usual method of soil sampling was unsatisfactory. Further investigations proved that there was a marked variation of soil reaction with depth, especially in the treated plots, and that reliable data could only be expected when the *pH* of each half-inch layer was determined separately.

The following method of obtaining solid plugs of soil for the *pH* estimations was evolved after some trial. The lid was removed from a cylindrical tin of about 2 in. diameter, the tin placed on the turf, bottom upwards, and "heeled in." The thin edge acted as an efficient cutter and it was found that if the tin was rotated a few times after being driven in, the soil column was effectively broken, and the tin could be withdrawn with a solid plug inside: with the lid replaced the tin could then be carried without risk of injury to the contents. In the laboratory the lid was removed and the bottom of the tin wrenched off to expose the turf. A cylindrical piece of wood was placed against the turf, and the lower end of the plug pushed out half an inch at a time and cut with a knife. In this way a marked degree of precision in cutting the layers was obtained, while the supporting walls of the tin prevented any crumbling during the process.

In order that the initial sample might be as comparable as possible with subsequent samples, and further to avoid errors due to soil heterogeneity, sample plugs from the experimental treated and control plots were taken from adjacent areas. The positions from which the initial samples were removed were carefully noted and subsequent samples taken within a few inches of the original positions.

## TRIALS BEGUN IN 1927.

*Stoke Poges Golf Club.*

The experiments at this course being of a preliminary nature, a résumé only of the results is here given.

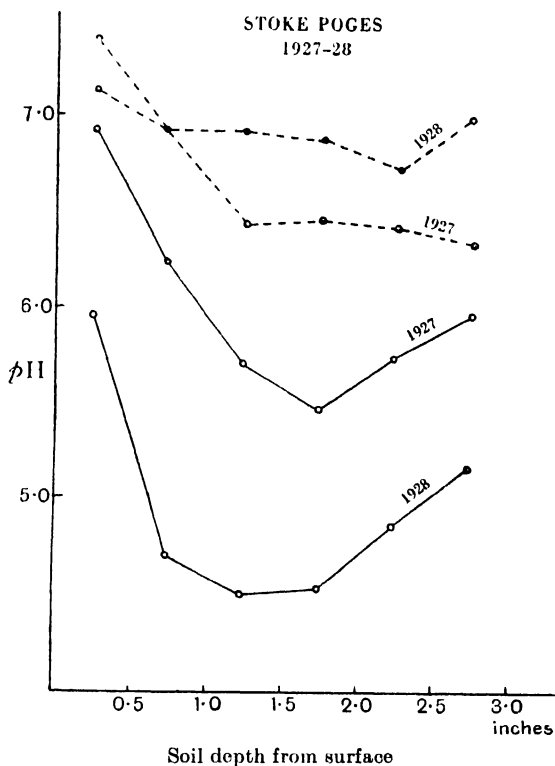


Fig. 1. The relationship between soil depth and soil reaction. The dotted lines represent untreated turf, while the continuous lines represent turf treated with periodic dressings of ammonium sulphate.

The trials consisted of direct comparisons between control areas and plots dressed with ammonium sulphate. The dressings were applied fortnightly from April to September at the rate of 5 lb./1000 ft.<sup>2</sup> To prevent the turf suffering by contact with solutions of high concentration, the treated areas were watered by means of a hose immediately after the applications. Three experiments in all were carried out, two on a neutral clay soil and one on an acid sand.

By the end of the first season (1927), the treatment had brought

about striking reductions in the areas covered by weeds. The diminutions were from an original 40 to 8 per cent. and from 23 to 5 per cent. on the clay, and from 17 to 0.5 per cent. on the sand. On the clay soil further smaller reductions took place in the second year (1928). The most susceptible weeds were *Trifolium repens*, *Achillea Millefolium*, *Prunella vulgaris* and various species of moss.

No reliable data on the changes in hydrogen-ion concentration in the soil were obtained until the final technique of soil sampling was adopted. The marked variation in the pH gradient shown in Fig. 1 is based on data from one of the clay-soil experiments. The curves marked "1927" represent plugs taken in the spring of 1928, the autumn samples being labelled "1928." The dotted line represents the control plot, while the ammonium sulphate treated plot is shown as a continuous line. On the presumption that the control and nitrogen plots were initially of a similar pH and that no great change had taken place during the year on the former, it will be seen that the first year's treatment has resulted in a marked increase of acidity, while a further decline in pH has occurred in the second year. The form of the curve has been altered from the "normal" straight line into one having a maximum acidity at 1.5 in. Increases in hydrogen-ion concentration were of the same order in the other trials, the maximum again occurring at a depth of 1-1.5 in.

These preliminary experiments established the fact that the treatment adopted in America was also satisfactory in England. The increasing soil acidity which was associated with weed reductions appeared to support the current view that acidity was the controlling factor in weed eradication.

#### TRIALS BEGUN IN 1928.

The positive results of the Stoke Poges trials in 1927 made confirmatory experiments on other golf courses desirable. Mr S. W. Cole, of the Biochemistry School of Cambridge University, kindly offered to collaborate, and it was by his help that trials were arranged at the Bishop's Stortford Golf Course and the Royal Worlington Golf Course, Mildenhall. In addition, Mr Cole undertook to devote his private lawn to more detailed investigations.

Owing to circumstances that could not be controlled, initial botanical analyses and soil sampling for pH determinations were omitted in all three experiments. In the case of two of the experiments, however, this omission was made good by the use of statistical methods in the analysis of the data.

*Bishop's Stortford Golf Course.*

This course is situated on boulder clay. The greens have been formed from the natural turf. The trial consisted of a strip 12 ft. wide running across a single green which received every fortnight ammonium sulphate applied as a dilute solution at the rate of 3 lb./1000 ft.<sup>2</sup> Owing to the dry summer and lack of watering facilities, the dressings were omitted on several occasions owing to the danger of scorching.

*Changes in botanical composition.* After the final dressings, four plots (10 ft. × 10 ft.) on the treated strip and four plots on the control area were selected at random for botanical analysis by the modified method. In Table I are shown the calculated mean areas covered by each species. In the third and sixth columns the odds against the differences being due to chance have been calculated according to Fisher(1), using his "table of *t*." When no figures are shown in the last column, the differences are not significant.

Table I.

*Botanical examination of the herbage.*

Species	Percentage area covered by species					
	Bishop's Stortford			Mildenhall		
	Treated	Control	Odds for significance	Treated	Control	Odds for significance
<i>Agrostis</i> spp.	78.75	62.9	100-1	6.4	5.0	—
<i>Poa</i> spp.	6.7	7.32	—	0.8	0.6	—
<i>Lolium perenne</i>	1.5	2.4	—	—	—	—
<i>Cynosurus cristatus</i>	0.05	0.075	—	—	—	—
<i>Festuca ovina</i> *	1.9	1.6	—	72.7	57.7	100-1
<i>Trifolium repens</i>	5.0	10.3	20-1	0.2	1.63	—
<i>Trifolium dubium</i>	—	0.1	—	—	—	—
<i>Achillea Millefolium</i>	1.05	8.69	20-1	4.4	14.9	100-1
<i>Sagina procumbens</i>	3.33	3.67	—	0.9	0.33	100-1
<i>Veronica serpyllifolia</i>	0.1	—	—	—	—	—
<i>Plantago media</i>	0.2	0.2	—	—	—	—
<i>Cerastium vulgare</i>	0.2	0.2	—	0.23	0.03	—
<i>Taraxacum officinale</i>	0.2	0.4	—	0.27	0.3	—
<i>Avena flavescens</i>	—	—	—	9.2	8.0	—
<i>Trifolium procumbens</i>	—	—	—	1.31	1.5	—
<i>Lotus corniculatus</i>	—	—	—	4.3	4.3	—
<i>Galium saxatile</i>	—	—	—	1.6	1.6	—
<i>Erodium cicutarium</i>	—	—	—	—	0.2	—
Moss	—	—	—	0.17	3.8	—
"Total weeds"	10.6	25.4	100-1	9.9	26.2	100-1

\* Under *F. ovina* are included all the fine-leaved fescues, which in some cases may be *F. rubra*.

The total weed figures show that there has been a reduction in weed area from 25.4 to 10.6 per cent. after one season's treatment. The most

striking changes in the individual weed species are the diminution of *A. Millefolium* and *T. repens*. Of the other species *S. procumbens* appears to be unaffected, while the remainder were present initially in too small quantities for any conclusions to be drawn. Of the grasses only *Agrostis* spp., with a marked increase, shows any significant changes.

It was found very difficult to separate completely, even with the aid of a lens, the different species of *Agrostis* and *Poa*. It was considered that the large amount of time that would be involved in identifying the species would not be justified, since the estimation by eye of the area covered by a grass species is far more liable to error than that of a weed species. This error is due to the morphological differences between weed and weed and weed and grass being so much greater than between grass and grass. The species of *Agrostis* found on all golf greens in this report consist of a mixture of *A. tenuis* and *A. stolonifera* according to Malte's classification (4). The commonest *Poa* species are *P. annua* and *P. trivialis*.

*Changes in soil reaction.* As at this centre no initial soil samples had been taken, plugs to a depth of 3 in. were first obtained at the conclusion of the experiment. These plugs were taken as usual 2 ft. from each side of the boundary line. The pH figures for the control and treated areas showed no significant differences on account of the initial high chalk content. The mean pH's of the six half-inch layers were 7.18 and 7.32 for the treated and control areas respectively.

*Royal Worlington Golf Course, Mildenhall.*

The soil on the green chosen for the experiment is of a peaty nature containing sand and chalk particles, while the flora is typical of the East Anglian breckland. The trial on the experimental green consisted, like that at Bishop's Stortford, of a 12-ft. strip running across the green, treated fortnightly with ammonium sulphate at the rate of 3 lb./1000 ft.<sup>2</sup> The dressings were occasionally omitted in the very dry periods on account of the scarcity of water.

*Changes in botanical composition.* Examination of the treated and control areas was first made in the autumn. Three plots of the usual size were chosen at random from the control and treated portions. The data obtained are shown in Table I on the same scheme of representation as in the Bishop's Stortford trial.

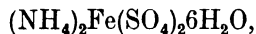
In the grass species, the only change under the treated is the increase of the dominant *F. ovina*. Of the weeds only one species, *A. Millefolium*, shows a significant decrease, but there are small differences in the other species which, though not in themselves significant, show in aggregate

a diminution in the "total weed" figures. The increase under treatment of *S. procumbens* is to be noted. The figures for *T. procumbens* and *G. saxatile* suggest that both these weeds are capable of successful growth under the conditions of treatment.

*Changes in soil reaction.* In the autumn, after the final dressing had been applied, plugs were taken with the usual precautions from the control and treated areas. The differences in the pH figures were not significant, the mean of the pH's of the control and treated areas being 7.47 and 7.42 respectively. Thus at the end of the experiment weed control was in course of establishment, despite the fact that the soil still showed no acid reaction.

*Private Lawn, Cambridge.*

The trials carried out on this lawn were of a more complex nature than those previously attempted. Part of the lawn had received during 1927 periodic dressings of ammonium sulphate and a reduction in the weed area had resulted. In 1928 on one portion, which had not as yet received any fertiliser dressings, three plots were laid out, each measuring 20 ft.  $\times$  20 ft. The first plot was dressed with ammonium sulphate at the rate of 3 lb./1000 ft.<sup>2</sup> weekly from March 25th to the end of September. The second plot received ferrous ammonium sulphate,



at a rate equivalent to the nitrogen given on the first plot, namely 10 lb./1000 ft.<sup>2</sup> every week from May 7th to the end of September, no fertiliser being given to the control plot. The use of ferrous ammonium sulphate was suggested by Mr Cole, who thought that some important principle might underlie the successful empirical use of ferrous sulphate in conjunction with ammonium sulphate, these being the common constituents of "lawn sand." The conditions of application, however, of "lawn sand" are to some extent different, in that much of the weed destruction is brought about by its "scorching" action, since the "sand" in practice is not watered in.

*Changes in botanical composition.* When the plots on the previously untreated turf were laid out, a rough estimation of the area covered by the total weeds was made on each plot. The object was to obtain plots in which the proportions of weeds to grasses should not be originally so divergent as to militate against the differences in the rate of weed elimination being comparable. With the exception of the rough estimation, no detailed observations could be carried out until June 11th. Subsequently three further analyses were made during the season.

In Fig. 2 the percentage area of the total weeds has been plotted against the time of treatment. The curve for the control plot shows that the weeds have remained substantially unchanged during the season. On the other hand, there is a very striking decrease on both the other plots, the ferrous ammonium sulphate being more effective than ammonium sulphate. If the initial points on these two curves are neglected, on account of the errors arising out of the determinations being only approximate, then over the same period from mid-June to October and with the same number of dressings, the weeds on the ferrous ammonium

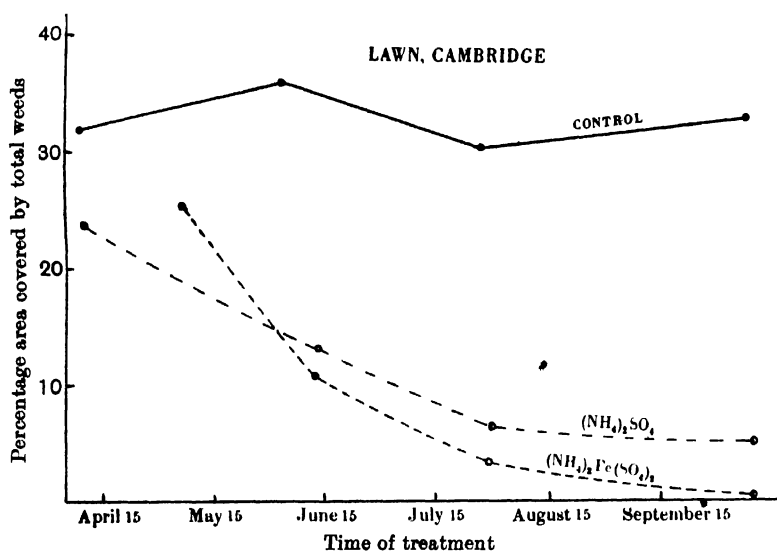


Fig. 2. Changes in the percentage area covered by the weed species in relation to duration of treatment.

sulphate plot have been reduced from 12.5 to 0.3 per cent. as against the 10.6-5.0 per cent. reduction in the ammonium sulphate plot. This difference would appear from the curves to be significant, but the data is insufficient for statistical verification. The lack of accurate initial data on the composition of each plot also precludes the calculations of the significance of the changes brought about by the treatments. The botanical changes, however, between June and October are shown in Table II.

On the control plot the figures show that the general composition is unchanged. The decrease in the clovers is rather to be expected, since there is generally a maximum development in June followed by a fall

in the autumn. The increase in the area of moss is probably due to the colonisation of the bare patches (decreased from 14.5 to 1.0 per cent.) under the more favourable autumn conditions. On the ammonium sulphate and ferrous ammonium sulphate plots, the changes in the proportions of *Agrostis* and *Poa* cannot do more than indicate that besides

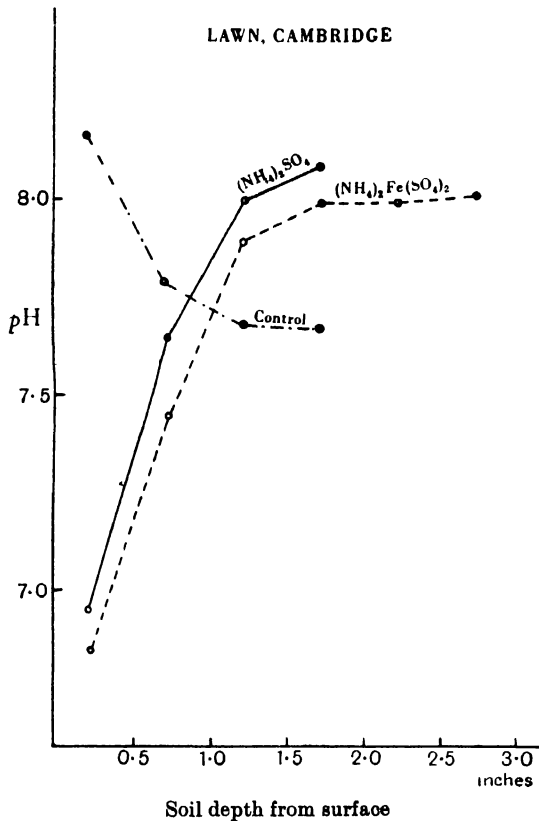


Fig. 3. The relationship between soil depth and soil reaction on control and treated plots.

the general increase there is perhaps a greater development of the *Poa* species. In the reductions of the weed species a similar trend is shown in both treatments, the double salt having the greater effect. The apparent increase of *T. repens* in the ammonium sulphate plot is probably due to experimental error.

*Changes in soil reaction.* At the end of the season plugs were removed from the plots and pH determinations carried out. The pH gradients for



the control, ammonium sulphate and ferrous ammonium sulphate plots are shown in Fig. 3. As initial *pH* data are not available, it is difficult to state definitely what changes took place during the season. Assuming that all the gradients were originally similar to the control, the two nitrogenous treatments would appear to have had the greatest effect in the first half inch of soil. Both ammonium sulphate and ferrous ammonium sulphate have lowered the *pH* in this layer to just below neutrality but the deeper soil is still alkaline. Confining attention, therefore, to the first 3 in. of soil, in which almost all the roots occur, the plant can be said to be growing in an alkaline soil.

Table II.

Species	Percentage area covered by species					
	Control		Sulphate of ammonia		Ferrous ammonium sulphate	
	June	October	June	October	June	October
<i>Poa</i> spp.	13.3	22.0	34.1	38.0	38.3	58.8
<i>Agrostis</i> spp.	31.5	36.9	53.2	58.0	48.4	41.4
<i>Festuca ovina</i>	4.4	4.5	—	—	—	—
<i>Trifolium dubium</i>	18.4	14.0	7.1	0.1	1.3	0.1
<i>Trifolium repens</i>	12.3	7.5	2.2	4.6	—	—
<i>Trifolium procumbens</i>	1.8	0.2	—	—	—	—
<i>Cerastium vulgare</i>	1.3	0.2	0.3	—	0.3	—
<i>Sagina procumbens</i>	0.9	0.2	1.2	0.3	—	—
<i>Achillea Millefolium</i>	0.3	0.15	0.2	—	—	—
<i>Veronica serpyllifolia</i>	—	—	0.8	—	0.3	—
Moss	1.2	11.7	—	—	—	—
Bare ground	14.5	1.0	—	—	—	—
"Total weeds"	36.2	33.75	11.65	5.0	12.5	0.3

## TRIALS BEGUN IN 1929.

The marked superiority of ferrous ammonium sulphate over ammonium sulphate in the 1928 experiment suggested that a mixture of ferrous and ammonium sulphates might be an efficient substitute for the double salt. On *a priori* grounds there should be little difference between the two types in their effects on soil or herbage. On the other hand, the cost of ferrous ammonium sulphate is four times that of the mixture. Accordingly, with the help of Mr Cole, an experiment was laid out on the Gog and Magog Golf Course.

*The Gog and Magog Golf Course, Cambridge.*

This course is situated only a few miles distant from the lawn on which the 1928 experiment was carried out. The conditions are similar, the soil being of the chalk type, the flora containing the same species.

On Green No. 7, a comparison was made between a mixture of ferrous and ammonium sulphates, ammonium sulphate and control plots. On Green No. 8 only ammonium sulphate and control plots were laid down. In both the experiments the ammonium sulphate was applied at the rate of 3 lb./1000 ft.<sup>2</sup>, while a part of Green No. 7 received in addition, mixed with the ammonium sulphate, ferrous sulphate at 6 lb./1000 ft.<sup>2</sup> The crystalline ferrous sulphate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) was used in this case, but the powdered calcined form ( $\text{FeSO}_4 \cdot \text{H}_2\text{O}$ ) was found to be more convenient and was employed at the proper equivalent rate in the other trials of 1929-30.

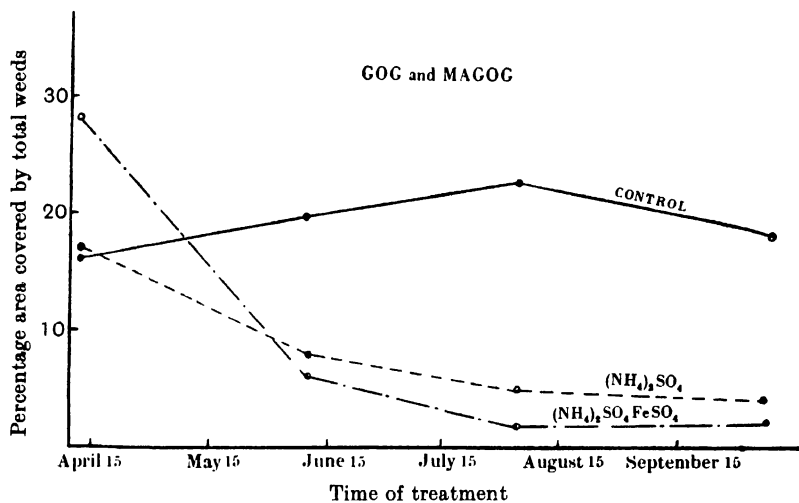


Fig. 4. Changes in the percentage area covered by the weed species in relation to duration of treatment.

(a) Green No. 7 was divided longitudinally into two halves, one acting as a control and the other receiving the ammonium sulphate. In addition, one-half of the treated portion was dressed at the same time with the ferrous sulphate. The applications were made from April to September at fortnightly intervals. Each dressing was, as usual, watered in, and, so far as is known, no "scorching" due to plasmolysis took place.

*Changes in botanical composition.* The changes in total weed area are illustrated in Fig. 4. The curves confirm the previous year's observations that the addition of ferrous sulphate to the ammonium sulphate renders the latter more effective, although this difference is not statistically significant. Here the weeds were reduced significantly from 27.9 to 1.8 per cent. by the combination as against a reduction from 17.0 to 3.7

per cent. in the plot receiving ammonium sulphate only. On the other hand, the percentage of weeds on the control area remained unchanged.

The differences between the initial and final composition of the turf under the various treatments is shown in Table III. In this table all changes in area covered which are statistically significant have been italicised.

Table III.

Species	Percentage area covered					
	Control		Ammonium sulphate		Ammonium sulphate, ferrous sulphate	
	12. iv. 29	1. x. 29	12. iv. 29	1. x. 29	12. iv. 29	1. x. 29
<i>Agrostis</i> spp.	72.6	62.4	70.2	69.8	49.7	68.7
<i>Poa</i> spp.	11.1	21.4	13.0	25.6	21.8	29.4
<i>Trifolium repens</i>	5.8	6.7	6.9	1.6	8.3	1.7
<i>Trifolium dubium</i>	7.9	5.2	5.9	0.0	12.4	0.1
<i>Achillea Millefolium</i>	0.3	5.6	4.0	1.9	12.4	0.0
<i>Cerastium vulgare</i>	0.5	0.5	—	—	—	—
<i>Ranunculus repens</i>	1.0	0.2	0.1	0.1	2.5	0.0
<i>Bellis perennis</i>	0.1	0.1	—	—	—	—
Moss	0.5	0.5	—	—	—	—
<i>Plantago media</i>	—	—	0.1	0.1	—	—
<i>Taraxacum officinale</i>	0.2	—	—	—	—	—
"Total weeds"	16.3	18.8	17.0	3.7	27.9	1.8

On the control area the only significant change has been the increase of the *Poa* spp., but the weeds on the whole also show a small increase. On the ammonium sulphate plot, the *Poa* spp. have again significantly increased, but on the other hand there has been a marked diminution in the principal weed species, although only the decrease of *T. dubium* is significant. With the addition of ferrous sulphate the weed reduction has become even more striking, the three dominant species all showing significant decreases. In the grasses, however, in contrast to the changes on the control and ammonium sulphate plots, the *Agrostis* spp. and not the *Poa* spp. have increased significantly.

*Changes in soil reaction.* Plugs to a depth of 3 in. were taken from all plots before any fertiliser was applied. At the end of the season other plugs were taken at a distance of 6 in. from the original samples. The pH gradients are shown in Fig. 5. The continuous lines represent the original pH gradients and the broken lines the gradients at the end of the season. In the control plot there are no changes with season in the first 2 in. but the third inch appears to have become less alkaline; this decrease in alkalinity also occurs in the other two plots and is there even more marked. Ammonium sulphate, both alone and in combination with ferrous sulphate, has reduced the pH value in the first half inch

by 0.27 and 0.21 respectively. When the first 3 in. of soil are taken together, weed control is seen to be in progress under alkaline conditions since the majority of roots confine their range to this layer.

(b) Green No. 8, like No. 7, was divided longitudinally into two halves, one-half only receiving ammonium sulphate at the usual rate.

*Changes in botanical composition.* Table IV shows the original and final botanical compositions of plots on the control and treated portions.

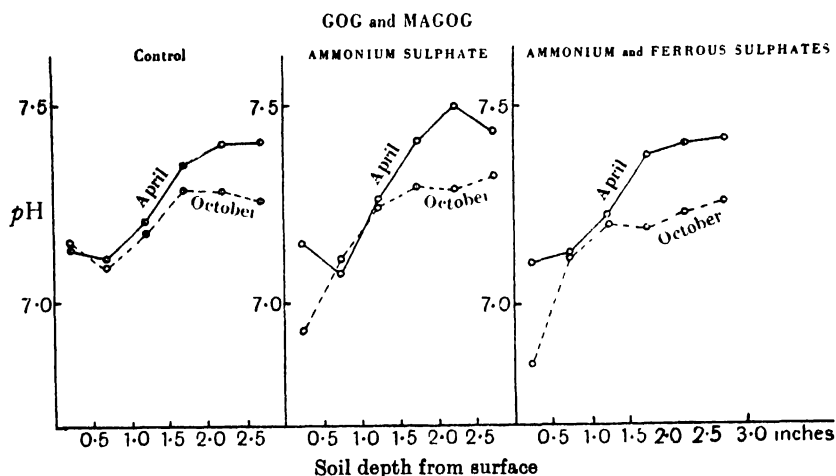


Fig. 5. The relationship between soil depth and soil reaction on control and treated plots.

Table IV.

Species	Percentage area covered					
	Treated			Control		
	13. iv. 29	1. x. 29	Odds for significance	13. iv. 29	1. x. 29	Odds for significance
<i>Agrostis</i> spp.	44.3	59.2	—	53.7	18.9	100-1
<i>Poa</i> spp.	4.8	22.4	100-1	3.8	7.9	—
<i>Festuca ovina</i>	1.0	2.1	—	0.5	9.7	20-1
<i>Achillea Millefolium</i>	41.1	15.5	100-1	36.9	55.2	—
<i>Trifolium repens</i>	5.6	0.7	20-1	3.5	7.2	—
<i>Trifolium dubium</i>	1.4	0.1	—	—	0.5	—
<i>Trifolium procumbens</i>	—	—	—	—	0.6	—
<i>Cerastium vulgare</i>	0.1	—	—	0.2	—	—
<i>Sagina procumbens</i>	—	—	—	0.1	—	—
<i>Taraxacum officinale</i>	0.5	—	—	0.02	0.3	—
<i>Plantago media</i>	0.1	0.1	—	—	—	—
"Total weeds"	48.7	16.3	100-1	40.9	63.5	20-1

The two plots show remarkably divergent changes, *A. Millefolium* on the control plot has increased from 36.9 to 55.2 per cent., whilst on the treated plot it has decreased from 41.1 to 15.5 per cent. This increase on the control plot is probably related to the summer drought, since the rich green colour of its leaves was in marked contrast to the scorched grass, suggesting that *A. Millefolium* is of a more xerophytic nature. On the control plot, *F. ovina* alone has gained ground, while in the treated area the *Agrostis* and *Poa* spp. have developed and *F. ovina* remained unchanged.

*Changes in soil reaction.* The changes in soil reaction brought about by the ammonium sulphate dressings are very small. On calculating the arithmetic mean of the pH value of the half-inch layers to a depth of 3 in., the initial values for the control and treated areas were found to be 7.42 and 7.53. Determinations of pH on the final samples showed that the control had not altered (7.45), while the fertilisers had lowered the pH value by a small amount (7.28). The botanical changes due to manuring therefore, once again seem to have taken place in an alkaline medium, without significant changes in soil reaction.

#### DISCUSSION.

Experiments extending over three years and on various types of soil have demonstrated that frequent periodic dressings of ammonium sulphate bring about a marked reduction in the weeds of closely cut turf. The behaviour of the weed species differs from individual to individual. The species most susceptible to nitrogen manuring were the clovers (*T. repens*, *T. dubium*) and *Achillea Millefolium*. It is difficult to be certain of the individual behaviour of other species since they were comparatively infrequent, but collectively they would appear to have decreased, with the possible exception of one or two seemingly resistant species, e.g. *Sagina procumbens*.

Although the weed changes were of a similar nature in all experiments, the grasses showed no such uniformity. In consequence the only general conclusion that can be drawn is that ammonium sulphate tends to favour the dominant species.

In four out of the five centres, the fertiliser brought about a marked reduction in the weeds without producing significant changes in the pH of the slightly alkaline soils. At the remaining centre the soil reaction was reduced from an initial figure somewhat below neutrality to approximately pH 5.0, but this degree of acidity was attained after the weeds had shown a marked diminution. It is thus impossible to accept the

view put forward in America that weed diminution is a result of soil acidification. Since the weed diminution observed is not associated with soil acidification, an explanation must be sought elsewhere. It might be suggested that the effect was a simple one of high nitrogenous manuring which affected the grasses more favourably and so enabled them to compete more successfully with the weeds. This view, however, is untenable for, as will be shown in a later paper, if the effects of a number of nitrogenous fertilisers are compared, weed reduction is confined to the ammonium salts. The alteration in botanical composition of turf which is brought about by such salts would thus seem to be due to the differential action of the ammonium ion on the plant population, that ion appearing to have a toxic action on the weeds while favouring the growth of the grasses.

The mode of action of ferrous sulphate in increasing the effect of ammonium sulphate is obscure.

#### SUMMARY.

The changes in botanical composition of closely cut turf (such as that of lawns) which result from the periodic applications of heavy dressings of ammonium sulphate have been followed at five centres during a period of 3 years. These dressings were applied at the rate of 3 lb./1000 sq. ft. (1.2 cwt. per acre) every 2 weeks during the spring and summer months. The applications were "watered in" to avoid injury from a solution of high concentration.

A method of botanical analysis by means of randomly selected quadrats was employed. The changes in botanical composition are expressed as changes in the area covered by each individual species. The data have been treated statistically where possible.

As a result of the treatment the areas covered by the weeds (*i.e.* all species other than the Gramineae) were greatly diminished. Some species such as *Trifolium repens* and *Achillea Millefolium* were more susceptible than others. As an illustration of the order of magnitude of this effect it was found at one centre that there was a decrease in "total weeds" (*i.e.* the sum of the areas covered by individual species) from 17.0 to 3.7 per cent., between April and September, of the total area examined. On the control plots the changes were slight.

A reduction in the "total weeds" was associated with an increase in the area covered by the grasses, the dominant species in each case showing the greatest expansion.

In addition to trials with ammonium sulphate, two experiments were carried out, one with ferrous ammonium sulphate and the other with an equivalent mixture of ferrous and ammonium sulphates. It was found that both these treatments produced greater reductions than ammonium sulphate alone. In one case ferrous ammonium sulphate at 10 lb./1000 ft.<sup>2</sup> reduced the "weed area" from 12.5 to 0.3 per cent., while ammonium sulphate applied at an equivalent nitrogen rate, reduced the area from 10.6 to 5.0 per cent. during the same period.

The changes of soil reaction show that the view that soil acidity is a pre-requisite for weed diminution by ammonium sulphate is untenable.

It is suggested that the effect of the ammonium sulphate is due to a differential action of the ammonium ions, which are toxic to the majority of the weeds while increasing the growth of the grasses.

The writer wishes to thank the Secretaries of the Bishop's Stortford, Royal Worlington, Gog and Magog and Stoke Poges Golf Clubs for their help, and also to express his gratitude to Sir Frederick Keeble and Mr Page for their suggestions. Finally, he is indebted to Mr Marmoy of Jealott's Hill for the bulk of the pH determinations.

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(Received August 19th, 1931.)

## THE OPERATION OF INTERSPECIFIC COMPETITION IN CAUSING DELAYED GROWTH OF GRASSES

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(With Plates X and XI.)

IN their researches on the sowing of land with grasses, Stapledon and Davies (14, 15) have drawn attention to the fact that in certain species the establishment<sup>1</sup> obtained shortly after sowing may be considerably smaller than that to be found some weeks or months later (cf. Table IV in reference (14)). Evidence was obtained indicating that this effect was due to the seeds of such species remaining dormant for considerable periods in the soil (*i.e.* to "delayed germination") and/or to the seedlings remaining in a condition of arrested development (*i.e.* to "delayed seedling growth"). It is implied in the latter assumption that such a delay in growth occurs before the seedling is sufficiently large to be identifiable. The following verbatim quotation summarises their conclusions on this point: "The data therefore show undoubted evidence of delay or latency either in the germination of sown seed or in the development of the young seedlings. There is no evidence available to show the proportions in which these two phenomena may have occurred, but on *a priori* reasoning it might be supposed that the critical seedling stages would not prove highly suitable to carry over a prolonged period of delayed growth" (15), p. 109).

Delayed germination has been found to occur in the seeds of a large number of species, but is generally associated with structural peculiarities that are not shown by the seeds of British grasses (cf. for example, Harrington and Crocker (3)); a number of cases occurring in the seeds of agricultural weeds are described by Nobbe and Haenlein (10).

The occurrence of delayed establishment was referred by Stapledon and Davies to conditions "adverse" to germination and growth, but no exact analysis of such conditions was made except with regard to the

<sup>1</sup> The term "establishment" is used throughout the present paper in the sense of meaning the number of seedlings sufficiently large to be referable to their species.



factor of interspecific competition. The results obtained by these workers showed very clearly that delayed establishment of several grasses (notably *Festuca pratensis*, *Phleum pratense* and *Poa trivialis*) was much more pronounced when *Lolium italicum* and *L. perenne* were sown along with them (cf. p. 117 in reference (15)). This competitive effect of the rye grasses is obviously of considerable practical importance as they are almost invariably included in commercial seed mixtures, and it is also of ecological interest; some insight into its exact operation would therefore seem desirable. The experiments described in the present paper have been carried out with the purpose of obtaining data to this end.

#### METHODS.

In the present investigation, attention has been confined to the behaviour, during and following germination, of four grasses, viz. *L. italicum*, *F. pratensis*, *Phleum pratense* and *Poa trivialis*. As stated above, the last three species have been found to show delayed establishment particularly markedly, while their seeds are very dissimilar and represent three distinct classes. The seeds of *F. pratensis* are amongst the largest of those of British grasses and carry much reserve food material; those of *Phleum pratense* are relatively small, but of high density; the seeds of *Poa trivialis* are still smaller in size and are extremely light with meagre food reserves. *L. italicum* has been found in the field to be the more active of the two rye grasses in causing suppression of other species.

The experiments fall into two groups: (a) those in which seeds of *L. italicum* were placed under conditions suitable for germination together with, and at the same time as, the seeds of each of the other grasses, the germination of the latter being compared with that given by them in the absence of the *Lolium*; (b) those in which seeds of *L. italicum* were sown earlier than those of the other species, so that the latter during germination were placed in competition with seedlings of rye grass; the germination given under such conditions was again compared with that obtained with no competition. The germination media employed were, in the case of group (a), moist discs of filter paper (such as are commonly employed at seed-testing stations) placed in closed Petri dishes; discs of agar-agar (2 per cent.) also in Petri dishes, the seeds being covered with the agar while it was still fluid; and completely sterilised soil in boxes. For the experiments in group (b), boxes of sterilised soil were again employed and seeds were also germinated in distilled water and in tap water containing soil. In all these cases

the conditions of experiment were under much greater control than can be exercised in the field, but they were at the same time very different from the conditions obtaining there; in this respect the experiments carried out in boxes of soil approximate most closely to the field environment. Also, the proximity of the seeds to each other in all the experiments was closer than that usual in the field.

Experiments were also performed to determine the behaviour of the four species of grass given above, towards different environmental factors; details of the methods employed can, however, most conveniently be described for each experiment separately.

Throughout the work the seeds used were taken from commercial samples.

## RESULTS.

### (a) *Experiments with coincident seeding.*

The results shown in Table I were obtained with seeds germinated on moist discs of filter paper in Petri dishes. Half the discs carried 100 seeds each of either *F. pratensis*, *Phleum pratense* or *Poa trivialis*; on the other half, 50 seeds of one of these grasses were alternated with 50 seeds of *L. italicum* on each disc; in both cases the seeds were set about  $\frac{1}{4}$  in. from one another. The dishes were exposed to the light in the laboratory and care was taken that the two series of dishes should be under the same conditions of temperature. The figures for *L. italicum*, *F. pratensis* and *Poa trivialis* represent averages from eight dishes in each case; those for *Phleum pratense* are averages from five dishes. All seeds showing either roots or shoots were counted as germinated. With all three species the germination occurring in the presence of seeds of *L. italicum* shows no significant difference from that obtained with *Lolium* absent, and there is no evidence for any peculiar competitive effect on the part of the seeds or seedlings of that grass.

Table I.

*Germination on moist filter paper of grasses in presence and absence of seeds of L. italicum.*

Species	% germination after 6 days		% germination after 11 days	
	<i>L. italicum</i> present	<i>L. italicum</i> absent	<i>L. italicum</i> present	<i>L. italicum</i> absent
<i>F. pratensis</i>	73.5	74.5	90.0	93.4
<i>Phleum pratense</i>	64.0	61.8	87.6	88.6
<i>Poa trivialis</i>	20.5	18.2	74.7	77.7
				15.2

The germination taking place in seeds immersed in agar-agar is given in Table II. Such seeds are under conditions of restricted aeration, and being in close proximity to one another (the seeds were set about  $\frac{1}{4}$  in. apart), would be expected to show any effect the presence of seeds of *L. italicum* might exert on account of respiration. No such effect is, however, revealed by the figures.

Table II.

*Germination in agar-agar of grasses in presence and absence of seeds of L. italicum.*

Species	% germination after 9 days		% germination after 38 days	
	<i>L. italicum</i> present	<i>L. italicum</i> absent	<i>L. italicum</i> present	<i>L. italicum</i> absent
<i>F. pratensis</i>	5.3	2.6	61.3	63.3
<i>Phleum pratense</i>	80.0	77.3	90.6	87.3
<i>Poa trivialis</i>	58.0	61.3	87.3	86.0

Table III summarises the behaviour of the seeds of the same three species when sown in sterilised soil; the experiments were, however, carried out at different times with the different species, under different conditions, and in slightly different ways. In each case the soil was contained in small boxes measuring  $8\frac{1}{2} \times 14\frac{1}{2} \times 3$  in., and, within each experiment, the whole of the soil used was very thoroughly mixed so as to ensure uniformity in all the boxes and the same weight and depth of soil placed below and above the seeds. Six such boxes were employed in each experiment, three being sown without *L. italicum* and three having seeds of that grass sown between rows of one of the other species. In every case the soil was kept at all times sufficiently moist for germination. The experiment on *F. pratensis* was carried out in June and in the open; ten rows, each of 20 seeds, were placed in each box, half the boxes therefore containing 200 seeds each of *F. pratensis*, and half having in each case 100 seeds of *L. italicum* placed in alternating rows with 100 seeds of *Festuca*. The seeds were about  $\frac{1}{3}$  in. from one another in rows  $1\frac{1}{2}$  in. apart and were covered with soil in each case to a depth of  $\frac{1}{2}$  in. The experiments on *Phleum pratense* and *Poa trivialis* took place during the winter and were carried out in a greenhouse kept at about 10° C. Similar boxes to those used with *F. pratensis* were employed in these cases, but 14 rows 1 in. apart and each containing 20 seeds were sown in each box and the seeds were only covered with  $\frac{1}{4}$  in. of soil.

Again the figures show no depressant action on the part of the *Lolium* towards the germination of the other grasses, and later experiments have

confirmed this result. It should be carefully noted that the conditions under which these experiments were performed were entirely favourable to the germination of all four species; furthermore, under such conditions the seedlings of all the species rapidly attained a size large enough to make possible their identification.

Table III.

*Germination in soil of grasses in presence and absence  
of seeds of L. italicum.*

Species		<i>L. italicum</i> present	<i>L. italicum</i> absent
<i>F. pratensis</i>	Germination (%) after 9 days	83.6	77.0
	" 42 "	89.6	87.8
<i>Phleum pratense</i>	" 13 "	66.0	60.0
	" 54 "	80.0	79.5
<i>Poa trivialis</i>	" 18 "	24.7	24.4
	" 44 "	60.2	58.8

(b) *Experiments with differential seeding.*

A number of experiments were carried out in which seeds of *L. italicum*, *F. pratensis*, *Phleum pratense* and *Poa trivialis* were germinated in water in Petri dishes and the seedlings allowed to grow on for at least 17 days. At the end of this time the seedlings were removed and fresh seeds placed in the dishes; the germination occurring in these seeds was compared with that occurring in similar seeds placed in water exactly comparable except that seedlings had not been grown in it. The results obtained are given in Table IV; the figures represent the average germination given by 600 seeds. No inimical effect on the part of the *Lolium* towards the germination of the other grasses is revealed.

Table IV.

*Germination of grasses in water, and in water in which  
seedlings had formerly grown.*

				%
Germination after 10 days of <i>F. pratensis</i>	In water in which	<i>L. italicum</i>	had germinated	84.0
	"	<i>F. pratensis</i>	"	88.5
	"	no seeds	"	75.5
Germination after 14 days of <i>Phleum pratense</i>	"	<i>L. italicum</i>	"	90.0
	"	<i>Phleum pratense</i>	"	86.5
	"	no seeds	"	85.0
Germination after 14 days of <i>Poa trivialis</i>	"	<i>L. italicum</i>	"	93.0
	"	<i>Poa trivialis</i>	"	93.0
	"	no seeds	"	94.0

In a further series of experiments the second lot of seeds was placed in the dishes along with seedlings of *L. italicum* 18 days old, which were

allowed to remain, and in comparable dishes in which no germination had occurred. In this case tap water was employed to which had been added 20 per cent. by weight of soil. The germination figures are given in Table V; those for *Phleum pratense* and *Poa trivialis* are averages of 600 seeds, those for *F. pratensis* averages of 500 seeds.

Table V.

*Germination of grasses in water containing soil, in presence and absence of seedlings of L. italicum.*

				<i>L. italicum</i> present	<i>L. italicum</i> absent
Germination (%) after 10 days of <i>F. pratensis</i>				87.5	88.0
"	8	"	<i>Phleum pratense</i>	60.0	68.5
"	31	"	<i>Phleum pratense</i>	85.0	83.0
"	9	"	<i>Poa trivialis</i>	38.5	84.0
"	23	"	<i>Poa trivialis</i>	72.5	89.5

The figures in Tables IV and V together are taken as showing definitely that seedlings of *L. italicum* do not produce any organic substance inimical to the germination of *F. pratensis* and *Phleum pratense*. In the case of *Poa trivialis*, however, there is a very well-marked depression of germination shown in the presence of the *Lolium* seedlings (cf. Table V). That this also is probably not due to any toxic excretion, but to the increased concentration of carbon dioxide resulting from the respiration of the seedlings, is shown by the fact that good germination occurs in water in which *Lolium* seedlings had formerly grown and by the increasing germination with time even in the presence of seedlings of that grass. This receives further confirmation also by the marked susceptibility to the presence of carbon dioxide shown by seeds of *Poa trivialis* when germinated in atmospheres containing that gas (cf. Table VIII).

Experiments having a similar principle to that of the above were carried out, using boxes of sterilised soil. Seeds of *L. italicum* were sown in the boxes in rows about 2 in. apart, the seeds being about  $\frac{1}{2}$  in. apart in the rows; boxes containing exactly comparable soil were also left unsown. After growth had proceeded for several weeks (the times for each experiment are given in Tables VI and VII), strips of soil of equal depth (approximately  $\frac{1}{2}$  in.) and weight within each experiment were removed, a second lot of seeds sown in the furrows, and the soil replaced. Such seeds, therefore, during germination were in close proximity to actively growing seedlings of *L. italicum*, and on account of the small size of the boxes (the dimensions were those given earlier) there was a very high density of roots in the soil. The boxes containing no seedlings

were sown in similar fashion; in both cases the soil was kept moist throughout the course of each experiment.

It will be seen from the figures given in Table VI, that seedlings of *L. italicum* of about 4 weeks in age have had no appreciable effect on the germination of the other three species of grass. If, however, the *Lolium* seedlings are allowed to attain a greater age, and consequently greater size, before the seeds of the other species are sown between them, a different result is obtained.

Table VI.

*Germination in soil of grasses in presence and absence  
of seedlings of L. italicum.*

		%
Germination after 10 days of <i>F. pratensis</i>	Between seedlings of <i>L. italicum</i> 5 weeks old	82.5
	Without seedlings	81.5
Germination after 20 days of <i>Phleum pratense</i>	Between seedlings of <i>L. italicum</i> 4 weeks old	73.5
	Without seedlings	78.0
Germination after 14 days of <i>Poa trivialis</i>	Between seedlings of <i>L. italicum</i> 5 weeks old	83.5
	Without seedlings	83.0

The figures given in Table VII show that in the presence of seedlings of *L. italicum* 2 months old, the germination of *F. pratensis* has been slightly depressed, and that of *Phleum pratense* and *Poa trivialis* very considerably so; consideration of the results of the earlier experiments allows of no other conclusion than that an increased concentration of carbon dioxide, consequent on root respiration, is the factor responsible for this effect.

Table VII.

*Germination in soil of grasses in presence and absence  
of advanced seedlings of L. italicum.*

		%
Germination after 24 days of <i>F. pratensis</i>	Between seedlings of <i>L. italicum</i> 8 weeks old	82.3
	Without seedlings	91.0
Germination after 20 days of <i>Phleum pratense</i>	Between seedlings of <i>L. italicum</i> 10 weeks old	40.0
	Without seedlings	89.0
Germination after 19 days of <i>Poa trivialis</i>	Between seedlings of <i>L. italicum</i> 8 weeks old	35.5
	Without seedlings	79.0

Nine weeks from the sowing of the seeds of *Poa trivialis*, the plants of *L. italicum* between which these seeds had been sown were cut away from the roots, so that no further growth could occur; at the same time the seedlings of *Poa trivialis* present were marked by pins. Twenty days afterwards there was an increased germination in the *Poa trivialis* of 11 per cent. (of the number of seeds sown); 11 weeks later the number

had increased to 18 per cent. In the case of the boxes in which *Phleum pratense* had been sown, which were treated exactly similarly the increased germination after 21 days was 2 per cent. of the seeds sown, and after a further 10 weeks 10.5 per cent. We have here, then, evidence for a certain amount of delayed germination in *Phleum pratense* and for a considerable amount in *Poa trivialis*, caused by the presence of plants of *L. italicum*. When, however, one considers, firstly, the long interval (at least 2 months) which had occurred between the sowing of the different grasses, secondly, the fact that the earlier sown grasses were growing in restricted root space so that the soil in which the second lot of seeds was sown was densely crowded with roots (at the time of sowing they were visible at the surface), and thirdly, the relatively small amount of germination that was delayed in *Phleum pratense* under these extreme conditions (and necessarily so also in the case of *F. pratensis*), it becomes extremely doubtful whether these data offer any explanation of results, obtained in the field. It seems in the highest degree unlikely that conditions bringing about such a very unequal state of competition can occur at all frequently in practice. Greater significance is attached to the behaviour of seedlings arising from sowings made under less intense competition.

Seeds of *F. pratensis* were sown in July between seedlings 6 weeks in age of *L. italicum* and of *F. pratensis*, growing in rows  $2\frac{1}{2}$  in. apart in the small boxes already described. After 23 days a germination of 90 per cent. between the *Lolium* seedlings and of 87 per cent. between those of *F. pratensis* had occurred, these being the maximum figures obtained. In both cases, after rapidly attaining a height of about  $1\frac{1}{2}$  in., the seedlings from this second sowing ceased to increase in size, and as fresh leaves were slowly formed the older ones withered, so that the seedlings carried no more than two or three small leaves at any one time. Although so small in size and although under extreme competition, these seedlings remained alive for a prolonged period. Three months from the date of sowing only 9 per cent. of the seedlings between *L. italicum* plants and 4.5 per cent. of those between *F. pratensis*, had died; 10 months from the date of sowing 71 per cent. and 79 per cent. respectively were still alive. These seedlings, although they had over-wintered, were no higher than they had been 2 weeks after germination, and had still only two or three functional leaves; it was not possible to determine to which species of grass they belonged and were such seedlings to occur in experimental field plots, they would not be included in any analyses of the composition of the pasture. The extreme difference in size between

seedlings which had been in competition with plants of *L. italicum*, and seedlings sown at the same time and grown under the same conditions in the absence of *Lolium*, is shown in Plate XI, fig. 1; the upper seedlings are those which have suffered suppression, those of the lower row are the controls. It should be mentioned that the latter, in consequence of growing crowded together in small boxes, are themselves suffering from the effects of competition and are far from normal in size; at the time of photographing, all the seedlings were  $10\frac{1}{2}$  months old.

A small number of *Festuca* seedlings which had been suppressed in development for  $10\frac{1}{2}$  months by plants of *L. italicum* were transplanted into fresh sterilised soil; all grew on into normal plants of *F. pratensis*, flowering occurring after some weeks.

An attempt was made to analyse the factors through which the competition was operative. Half the rows of older dominant plants were cut almost to the ground level in each of the boxes which had grown for 10 months as described, so that competition for light with the suppressed seedlings growing between was removed, the cutting being repeated at intervals when made necessary by the growth of the older plants. Three days later, half the boxes were given a solution containing 0.15 per cent. potassium dihydrogen phosphate and 0.15 per cent. ammonium nitrate, and this solution was supplied every 2 or 3 days. The suppressed seedlings could now be separated into four different lots growing under four different environmental conditions, viz. (a) competing with the older seedlings for nutrients but not for light (row A in Plate X, fig. 1); (b) under competition for both nutrients and light (row B in fig. 1); (c) competition for light and nutrients removed, root respiration being the only competitive factor acting on the seedlings (row C in fig. 1); (d) competing with the older seedlings for light, but not for nutrients (row D in fig. 1). The differences in behaviour under these various conditions is well shown in Plate X, fig. 1, in which all the seedlings illustrated are 14 months old and have received the different treatments for 15 weeks. At the end of this time the seedlings receiving both nutrition and light were the only ones large enough for identification, and these had made such rapid growth that it was clear that even under these extreme conditions, root respiration had been a negligible factor in competition; here also it should be mentioned that towards the end of the period competition for light was occurring between the resuscitated seedlings themselves.

The presence of either light or nutrients alone was equally ineffective in causing any marked recovery in the seedlings, but the two factors



had very different effects. While those seedlings receiving light alone were still very small, they had a greater assimilatory surface than the seedlings competing for both factors, and were obviously capable of an extended survival; actually, they afterwards showed a slow increase in size. Those seedlings receiving nutrients alone, on the contrary, while showing a greater increase in size at the commencement, were all dead at the end of the 3 months, and this lack of persistence has been shown, in similar experiments, by seedlings receiving higher nutrition from the commencement of germination. At the time of photographing, the three seedlings shown in row D in the figure were, therefore, dead, but they show the increase in size that had taken place previous to death. It should be noticed in this experiment that plants of *F. pratensis* behaved in exactly similar fashion to those of *L. italicum* in suppressing the growth of later sown seedlings.

Throughout the experiment, and also in those described later on *Phleum pratense* and *Poa trivialis*, competition for water was eliminated, as this is not believed to occur at all frequently in the field. It should, however, be stated that observations on seedlings growing in small boxes have indicated that relatively small plants of *L. italicum* (not more than 4 weeks old) transpire sufficiently rapidly to have an appreciable effect on soil moisture.

Delayed growth of seedlings has also been found to occur in *Phleum pratense* and *Poa trivialis*, and is the more remarkable in these grasses in that the seedlings are so minute. Seeds of *Phleum pratense*, sown between seedlings of *L. italicum* 4 weeks old, gave a germination of 73.5 per cent.; 10 weeks from the date of sowing the same number of seedlings was present. They were from 1 to 2 in. in height and had two or three very thin, small leaves; such seedlings 13 weeks old are shown in the upper row of Plate XI, fig. 2, they show a great variation in size from the seedlings of the lower row which were of the same age and grown under the same conditions, but in the absence of *Lolium*. Suppressed seedlings 13 weeks old, when transplanted into fresh soil, grew rapidly and flowered. Fifteen weeks after sowing, 3 per cent. of the seedlings between plants of *L. italicum* had died; between comparable plants of *Phleum pratense* the mortality in the same period was likewise 3 per cent. The boxes were now treated as in the case of *F. pratensis*, half the older plants in each box being kept cut down and half the boxes being given a nutrient solution. Exactly similar results were obtained, the seedlings receiving both nutrients and light making good growth; those receiving light alone, very little; and those receiving

nutrients only, dying after an attempt at growth. Seedlings 6 months old which had received the four different treatments for 10 weeks, are shown in Plate X, fig. 2.

Although, here also, plants of *Phleum pratense* had a similar effect to those of *L. italicum* in suppressing the growth of later sown seedlings, the effect was not nearly so extreme, seedlings growing between plants of *Phleum* attaining a considerably larger size than seedlings between *L. italicum*.

In the experiments with *Poa trivialis* the results have been complicated by the occurrence of a good deal of delayed germination in the seeds of that species, so that it becomes difficult to know the exact age of any seedling examined. The seedlings shown in Plate XI, fig. 3, however, are all at least 11 weeks old, and probably older (5 months). The extreme smallness (in some cases  $\frac{1}{2}$  in. in height) of the seedlings which have been suppressed by plants of *Lolium*, makes their persistence more remarkable than in the case of either *F. pratensis* or *Phleum pratense*, yet in the case of the seedlings illustrated, during the last 11 weeks of the 7 months which had elapsed from the time of sowing the mortality was only 4 per cent.

When seeds of *Poa trivialis* are sown between older plants of the same species, the latter have a similar effect (but in a lesser degree) to plants of *L. italicum* in retarding the development of the later sown seedlings. Experiments, having regard to light and nutrition, exactly similar to those described for *F. pratensis* and *Phleum pratense* have been carried out with *Poa trivialis* and have yielded similar results. It is clear, therefore, in the case of all three species that the competitive action of the *Lolium* plants operates in two ways, viz. by withholding soil nutrients from the younger seedlings and by lessening considerably the illumination the latter receive. There is also evidence that the condition of low nutrition which results, assists the survival of the seedlings under feeble illumination; in this connection, the work of Gregory<sup>(2)</sup> on respiration and assimilation in plants of barley subject to mineral deficiency is of interest.

In these phenomena of persistence and recuperation in seedlings, we have clearly a possible explanation of the results obtained by Stapledon and Davies, to which reference has already been made; but it is necessary to assume a differential behaviour on the part of the seeds or seedlings of *L. italicum* as compared with that of the other grasses when sown in the field, in order that the former should obtain the initial advantage of growth which was accorded to plants of *L. italicum* in the experiments

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described above. Some evidence as to the way in which this can be brought about is provided when one considers the reactions of the several species, during germination, towards certain environmental factors.

### THE EFFECT OF DIFFERENT PHYSICAL FACTORS ON THE GERMINATION OF GRASSES<sup>1</sup>.

#### (a) *Aeration and carbon dioxide concentration.*

Seeds of *L. italicum*, *F. pratensis*, *Phleum pratense* and *Poa trivialis* were set to germinate on discs of filter paper soaked in distilled water. The discs were placed in Petri dishes (without covers) and these in turn in desiccators, sealed with vaseline, the atmosphere in which contained varying quantities of carbon dioxide; the concentrations used of that gas are given in Table VIII, they are, however, only approximate and there is, moreover, a possibility of leakages in vessels sealed in such a way; these considerations, however, do not affect the validity of the figures as a comparison between the species, since for the same concentration of carbon dioxide the seeds of all were contained in the same vessel. The desiccators were placed in the light and were subject to a temperature range of from 14.5 to 24° C.

Table VIII.

*Germination of grass seeds in different concentrations of carbon dioxide.*

			In air	In 5 % CO <sub>2</sub>	In 12 % CO <sub>2</sub>	In 25 % CO <sub>2</sub>
Germination (%) of <i>L. italicum</i>	after	7 days	85.0	80.0	73.5	1.0
" "	" 15 "	"	86.5	87.5	82.0	73.5
" <i>F. pratensis</i>	" 7 "	"	90.0	74.0	72.0	1.0
" "	" 15 "	"	95.0	92.0	89.5	51.5
" <i>Phleum pratense</i>	" 7 "	"	88.5	83.0	81.5	Nil
" "	" 15 "	"	90.0	86.5	86.5	19.0
" <i>Poa trivialis</i>	" 7 "	"	90.0	60.0	54.5	Nil
" "	" 15 "	"	93.5	87.5	76.0	Nil

The figures given in Table VIII (which are all averages of 200 seeds) show *L. italicum* to be the least affected in germination by the presence of carbon dioxide of the four species, while *Poa trivialis* is very decidedly the most susceptible. With all four species seeds that had been prevented from germinating by the presence of carbon dioxide germinated immediately that gas was removed, no induced dormancy, such as that found in seeds of *Brassica alba* by Kidd<sup>(6)</sup>, occurring.

<sup>1</sup> An exhaustive survey of the literature bearing on this subject is to be found *Keimungsphysiologie der Gräser* by Lehmann and Aichele, 1931.

The response of the four species of grass to restricted aeration is, however, different from that towards carbon dioxide concentration. The results given in Table IX were obtained from seeds submerged beneath different thicknesses of agar (2 per cent.), which had been poured over the seeds and allowed to solidify; the seeds had previously been soaked overnight in tap water, so as to avoid displacement by their floating in the fluid agar (an exactly similar experiment in which the seeds were not soaked yielded confirmatory results); throughout the period of the experiment the seeds were exposed to the light and to a fluctuating temperature. In this case, seeds having shoots were alone counted as germinated; the figures in the table represent averages of the germination occurring in 200 seeds.

Table IX.

*Germination of grass seeds immersed in 2 per cent. agar-agar.*

		Under 1.5 mm. depth of agar	Under 3 mm. depth of agar	Under 5 mm. depth of agar
Germination (%) of <i>L. italicum</i>	after 10 days	35.5	2.5	Nil
" "	" 26 "	85.0	77.0	69.5
" <i>F. pratensis</i>	" 10 "	5.0	Nil	Nil
" "	" 26 "	50.0	28.5	16.0
" <i>Phleum pratense</i>	" 10 "	80.5	63.0	35.0
" "	" 26 "	87.0	80.5	83.5
" <i>Poa trivialis</i>	" 10 "	34.5	24.0	Nil
" "	" 26 "	72.0	73.5	75.5

It will be seen that under the conditions of the experiment the germination of the larger seeded grasses is relatively much poorer than that of the smaller seeded, presumably on account of the greater respiratory exchange of the former. Of all the species the germination of *F. pratensis* is most adversely affected and that of *Phleum pratense* least.

The results summarised in Tables VIII and IX provide some insight into what is likely to be the behaviour of these seeds in the field. The susceptibility of the seeds of *Poa trivialis* to excessive amounts of carbon dioxide suggests the possibility of their germination being retarded by root respiration and this has been seen to occur in the experiments previously described. At the same time seeds so retarded are able to germinate, immediately the concentration of carbon dioxide falls, without the operation of any other factor. On the important question as to the possibility of seeds being kept dormant in the soil on account of poor aeration (*e.g.* by their being buried too deeply or their being in water-logged soil) it can be said, from the data of Table IX, that this is only

likely to occur in the case of *F. pratensis*. In actual fact, however, it has been found that the germination of all the grasses is good even when they are covered by a depth of soil greater than is ever likely to obtain under field conditions.

Seeds of the four grasses were placed in thin films of agar covering the bases of glass dishes, and the latter were filled with fine, sterilised soil, containing 20 per cent. moisture, to a depth of 2 in., the soil being pressed firmly together. With this arrangement, it was possible to observe through the bases of the dishes the germination taking place; also, by raising those dishes which contained seeds of *Poa trivialis*, a certain amount of light was admitted to the seeds. The germination of the several species was as follows: *L. italicum* 72 per cent.; *F. pratensis* 73 per cent.; *Phleum pratense* 66 per cent. and *Poa trivialis* 69 per cent. It should be noted that none of the seedlings of *Phleum pratense* and *Poa trivialis* reached the surface of the soil, and only 6 per cent. and 3 per cent. respectively of those of *L. italicum* and *F. pratensis*. With a similar arrangement, using 1 in. depth of soil, the number of seedlings visible at the surface, expressed as a percentage of the number of seeds sown, after 18 days at 15° C. was: *L. italicum* 84 per cent.; *F. pratensis* 81 per cent.; *Phleum pratense* 31 per cent. Even with a covering of only  $\frac{1}{2}$  in. of soil a number of seedlings of *Phleum pratense* failed to reach the surface and it is clear that any greater depth of covering than this must be detrimental to the establishment of this grass. More extensive data on this point have been obtained by Williams<sup>(16)</sup>, but those given above show that deep burial of seeds is inimical on account of the killing of seedlings rather than on that of causing delayed germination.

#### (b) *Light.*

Nelson<sup>(9)</sup> in his study of germination in different species of *Poa* found *Poa trivialis* to be comparatively indifferent either to the presence of light or to a fluctuating temperature. The results obtained with this grass by the present writer have been somewhat different and it would seem possible that the behaviour of different samples is not the same. Jönsson<sup>(5)</sup> found the reaction of the seeds to light to be dependent on age, old samples being the most indifferent to its presence.

Seeds of *Poa trivialis* on moist discs of filter paper in Petri dishes gave the following germination after 22 days: in the light, 72 per cent.; in darkness, 19 per cent. The dishes were kept on the bench of the laboratory and were subject to a temperature range of from 5 to 17° C., but the fluctuations in temperature did not take place sharply. When

light was excluded and the seeds kept at a constant temperature of 20° C., the germination was likewise poor, being after 23 days, 22 per cent. in a sample 15 months old, and 2.5 per cent. in one 3 months old. Subjected to a temperature of 20° C. for 17 hours in each day and of 27° C. during the remainder, the same two samples of seed gave, after 12 days, respectively 48 per cent. and 75 per cent. germination in complete darkness. The beneficial effect of light is not operative, however, solely through the production of a change in temperature, since seeds kept at a constant temperature of 27° C. have given, after 7 days, germinations of 45 per cent. and 64 per cent. in the light, compared with 22.3 per cent. and 19 per cent. in the dark (cf. (5)). Experiments have also shown that a beneficial effect of light is exerted on seeds buried in the soil, at least to a depth of  $\frac{1}{4}$  in. It is clear, therefore, that the depth to which seeds of this grass are sown will affect germination through the operation of this factor; there is also evidence that should the temperature over a period remain fairly constant, seedlings of other grasses may retard the germination of *Poa trivialis* on account of their decreasing the intensity of illumination to the soil.

At low temperatures, the germination of *Phleum pratense* may also be higher in the light than in the dark; in one experiment having a temperature range of 0–12° C., the germination of this grass occurring on discs of filter paper exposed to the light was 41 per cent. and on similar discs with light excluded 15 per cent., in both cases after 58 days<sup>1</sup>. A beneficial action by light on germination in this grass was observed by Kling (7) to occur at 20° C., but the results of other workers have not been confirmatory (Jönsson (5), Pieper (12)).

Kreysing (8) found recently harvested seeds of *F. pratensis* to give an increased germination in the light; older samples were indifferent to this factor. At an average temperature of 5.5° C., the seed of this species used in the present work showed no response to exposure to light.

### (c) Acidity.

The germination of the different species of grass that takes place in four concentrations of sulphuric acid is shown in Table X, the figures being averages of 600 seeds. The seeds were floated on the surface of the acid in Petri dishes and the seeds of *Phleum pratense* and *Poa trivialis* were placed in the same dishes as the seeds of *L. italicum* and *F. pratensis* in order to minimise any effect due to the neutralisation of the acids by adsorption in the glumes of the larger grasses.

<sup>1</sup> Seeds of *Dactylis glomerata* have also been found to behave similarly.

Table X.

*Germination of grass seeds in different concentrations  
of sulphuric acid.*

Germination (%) of:			Distilled H <sub>2</sub> O	0.0025 N H <sub>2</sub> SO <sub>4</sub>	0.005 N H <sub>2</sub> SO <sub>4</sub>	0.0075 N H <sub>2</sub> SO <sub>4</sub>	0.01 N H <sub>2</sub> SO <sub>4</sub>
<i>L. italicum</i>	after	6 days	74.0	79.0	28.0	6.0	0.5
"	"	13 "	93.0	92.0	64.5	18.0	4.0
<i>F. pratensis</i>	"	6 "	65.0	61.5	10.0	Nil	Nil
"	"	13 "	83.0	86.0	58.0	8.6	Nil
<i>Phleum pratense</i>	"	6 "	69.5	73.0	8.5	Nil	Nil
"	"	13 "	85.0	86.5	38.0	1.6	Nil
<i>Poa trivialis</i>	"	6 "	10.0	17.5	Nil	Nil	Nil
"	"	13 "	61.0	66.0	33.6	2.0	Nil

The figures are not considered to have any practical significance beyond exemplifying once more the greater resistance of the seeds of *L. italicum* towards factors inimical to germination.

(d) *Moisture.*

Seeds of *L. italicum*, *F. pratensis* and *Phleum pratense* were sown in sand which had been passed through a sieve of 1 mm. mesh and to which a known amount of water had been added. The seeds were covered in each case by approximately 6 mm. depth of sand and were kept in a closed incubator at 22° C. The percentages of moisture given in Table XI are of the total weight of the sand plus water mixture; the germination figures represent the number of seedlings visible at the surface of the sand and in each case are averages obtained from 250 seeds.

Table XI.

*Germination of grasses in sand of differing water content.  
Percentage germination after 9 days.*

Species	2.5 % moisture	1.25 % moisture	0.5 % moisture
<i>L. italicum</i>	67.0	28.0	Nil
<i>F. pratensis</i>	73.0	69.5	"
<i>Phleum pratense</i>	76.0	69.5	"

The figures in Table XI show *L. italicum* to have the highest water requirement of the three species; the closely related *L. perenne* has also been found to require much moisture(4). It is probable that the effect is due to the actual quantity of water required by the seeds of *Lolium* before germination will occur, since Pammer(11) found their ability to absorb water from sugar solutions (*i.e.* their suction pressure) to be at least as high as that of *F. pratensis* and *Phleum pratense*.

It would, therefore, seem that dryness of soil at the time of sowing in the field, would be relatively most detrimental to the germination of *Lolium* sp.; whether, however, the seedlings of the different species would show a similar relationship is not known.

(e) *Temperature.*

In order to obtain some knowledge of the reaction to low temperatures of the same four species of grass, the following very simple experiment was carried out. Seeds were placed on moist discs of filter paper in Petri dishes and the latter placed in a cupboard out-of-doors (to which light had access) during the months of December, January and February. The temperature within the cupboard, during the period of the experiment, was never higher than 12° C. or lower than 0° C., the average being about 5.5° C. The germination figures are given in Table XII, being in every case averages obtained from 300 seeds; seeds showing either roots or shoots were counted as germinated.

Table XII.

*Germination of grass seeds on moist filter paper at an average temperature of 5.5° C.*

Germination (%) of		After 24 days	After 58 days
"	<i>L. italicum</i>	80.0	86.5
"	<i>F. pratensis</i>	39.0	57.0
"	<i>Phleum pratense</i>	34.5	41.0
"	<i>Poa trivialis</i>	38.0	52.0

There is a very marked difference shown in the germination of *L. italicum* and that of the other three grasses which is much inferior. We have here, then, a factor (*i.e.* low temperature) which is capable of causing a differential germination in favour of *L. italicum* and therefore of bringing about such a state of unequal competition as obtained in the box experiments described earlier. A good example of this phenomenon occurred in boxes of sterilised soil which were sown with seeds of *L. italicum* and *Phleum pratense* in alternate rows and placed outside in the month of November. Thirty-nine days after sowing, 80 per cent. of the seeds of *L. italicum* had germinated (meaning in this case appearance above the soil) and only 11 per cent. of those of *Phleum pratense*. No further germination occurred in the latter until April, the germination on May 2nd (24 weeks after sowing) being 46 per cent., which, 5 weeks later, had increased to 56 per cent. In this case the *Lolium* gained no advantage by its earlier germination as most of the seedlings were killed during the winter; there is, however, reason to believe that, under more



normal conditions, not only is the germination of *L. italicum* relative to that of the other grasses favoured by low temperatures, but that the latter has a differential effect on growth which is again in favour of the *Lolium*.

It is evident, therefore, that should low soil-temperatures prevail at the time of sowing in the field the presence of *L. italicum* would be likely to induce suppression of the seedlings of grasses such as *F. pratensis*, *Phleum pratense* and *Poa trivialis*, with the possibility of these seedlings recovering and giving an increased establishment should the growth of the *Lolium* receive a check at a later date. Data would in this way be yielded similar to those obtained by Stapledon and Davies. As already stated, those authors made reference to the competitive effect of *L. italicum* being most pronounced when sowing took place under "adverse conditions," and from the results of the present experiments it is concluded that emphasis should be placed on low temperatures as being in that category.

Another factor which may operate in the field to the detriment of *Phleum pratense* and *Poa trivialis* is believed to be the behaviour of their seeds at the time of sowing. Experiments in the laboratory suggest that the seeds of these grasses, on account of their small size, pass more readily into the soil than do those of *L. italicum*, and there is thus a possibility of their being buried more deeply; should this occur, the seedlings of *L. italicum* would almost certainly be differentially favoured in development.

It is perhaps necessary to emphasise that although the experiments described above provide an explanation of the data obtained by Stapledon and Davies regarding delayed establishment, the evidence for the occurrence of that phenomenon in the field rests entirely on the results of those workers. At the same time, it is improbable, that, out of the wide range of conditions occurring in the field, some should not be found reproducing in all essentials the conditions shown herein to cause delayed growth of seedlings.

#### DISCUSSION.

The demonstration which has been given in this paper of the potentiality in the seedlings of certain grasses for prolonged persistence under conditions rendered unfavourable to their growth by the competition of other plants, and for rapid recovery on the removal of such conditions, carries with it implications of rather wide significance.

The importance of germination as a factor determining survival in competition between different species of grass has been clearly shown

by the critical experiments of Clements *et al.* (1) and the data obtained by Stapledon and Davies show the value of the vigorous germination that characterises *L. italicum*, in securing the rapid establishment usual with that grass. The results herein described, however, show that it is possible for certain species of grass ultimately to attain satisfactory establishment following on little or no early germination, as the persistence of their seedlings enables them to await the arrival of conditions suitable for their development. It is obvious, also, that this phenomenon must play an important part under natural conditions, not only in the colonisation of denuded areas but also in the invasion by alien species of land already occupied by other plants. In this connection, latency in seedlings must help to a considerable extent in bringing about the ecological changes which follow on the grazing and other activities of animals.

Salisbury (13) has recently drawn attention to the high rate of mortality in seedlings as compared with that occurring among adult plants. The results of the above experiments in no way conflict with his observations, but it is interesting to find that in certain plants, under certain conditions, that stage in their development which is relatively the most susceptible of mortality may none-the-less, in the absolute, be extremely tenacious of life. It is not known whether seedling-persistence is shown by any dicotyledons—field observations suggest that it may occasionally occur in *Trifolium repens*—and exact data on this point would be of interest.

The author feels considerable diffidence in remarking at all on the significance to practical agriculture of the work described in this paper, and the following comments are put forward quite tentatively, as being made by one who has little experience of the conditions prevailing in the field.

The observation of Stapledon and Davies that the sowing of mixtures of grass seeds containing *L. italicum*, under conditions adverse to germination, results in an increased relative establishment of that grass is readily understandable from the above data, and since the differential effect of low temperatures on germination is particularly well marked, the sowing of such mixtures early in the year would not seem advisable.

Paradoxical as it may seem, the above experiments suggest that high soil fertility may be detrimental to the establishment of the sub-dominant grasses should an unequal state of competition favouring *L. italicum* have been brought about by the operation of some other factor, since it has been seen that mortality in suppressed seedlings is

far higher with increased nutrition. The interesting possibility is offered also, of sowing on poor land a seed mixture containing *Lolium* (in this case as early in the year as required), grazing off the growth made by that grass during the early summer and of obtaining a satisfactory establishment of the suppressed species by applying fertilisers at a later date.

The few observations given above on the ability of the seedlings of different grasses to emerge through a covering layer of soil indicate that with the usual practice of sowing and harrowing in the field, the small seeded grasses are placed at a disadvantage compared with the large seeded species. Under conditions of moderate fertility it would also seem desirable to place the seedlings of *L. italicum* at a disadvantage as compared with those of the other grasses. Both these considerations would be met by placing the seeds of *L. italicum* deeper in the soil than those of the small seeded species. This would be the case were the larger seeds (such as those of *L. italicum*, *L. perenne* and *F. pratensis*) sown first, the field harrowed and such seeds as those of *Phleum pratense* and *Poa trivialis* sown on the surface to be followed only by rolling. It has to be admitted, however, that the results from surface sowing obtained by Williams<sup>(16)</sup> were far from satisfactory.

Finally, it is clear that in the sowing down of grassland, the extreme persistency and recuperative ability of grass seedlings provide the opportunity for achieving success by later management should the early results be poor.

#### SUMMARY.

1. An explanation is provided of the causation by *L. italicum* of delayed establishment in *F. pratensis*, *Phleum pratense* and *Poa trivialis*.

2. The presence of seeds or seedlings of *L. italicum* is not detrimental to the germination of the other species under conditions thought likely to occur in the field, except in the case of *Poa trivialis*, the seeds of which grass are extremely susceptible to the presence of carbon dioxide and hence to root-respiration.

3. Under certain conditions, seedlings of *L. italicum* inhibit completely the growth of seedlings of the other three grasses, the latter being deprived of light and soil nutrients. At the same time, seedlings suffering suppression in this way are remarkably persistent and are capable of immediate recovery on the removal of competition.

4. The significance of this behaviour, ecologically and to agricultural practice, is discussed.

5. The effect of different physical factors on germination in the same four species of grass is described. The action of low temperatures in depressing the germination of *F. pratensis*, *Phleum pratense* and *Poa trivialis* to a greater extent than that of *L. italicum* is considered an important factor in interspecific competition.

The author's sincerest thanks are due to Prof. R. G. Stapledon for suggesting this study and for his continued interest in the work.

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# EXPLANATION OF PLATES X, XI.

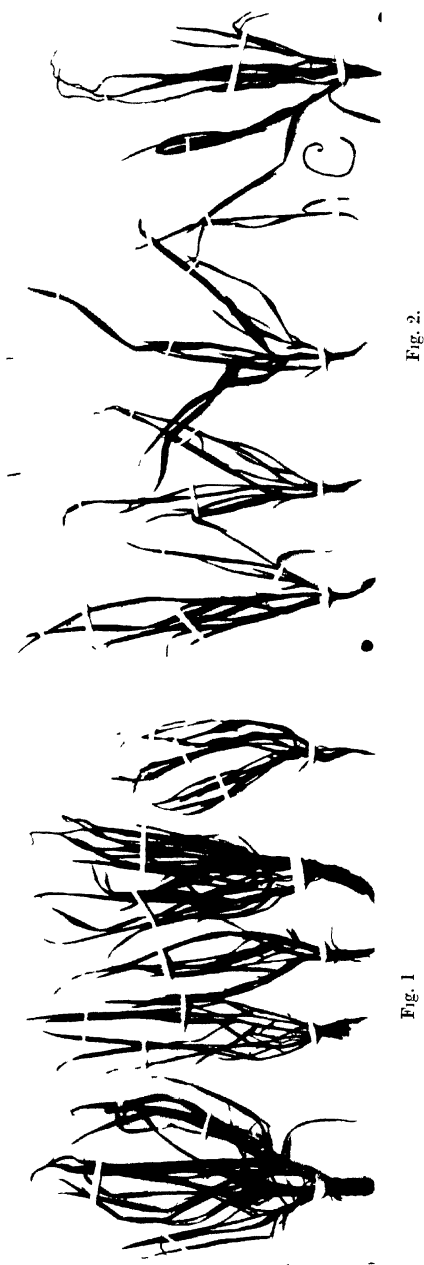
## PLATE X.

- Fig. 1. Seedlings of *F. pratensis* 14 months old which have been in competition with plants of *L. italicum*, but over a period of 15 weeks have grown under the following conditions: Row A. Receiving adequate light but mineral nutrition removed by plants of *L. italicum*. Row B. Deprived of both light and mineral nutrition by plants of *L. italicum*. Row C. Receiving adequate light and mineral nutrition. Row D. Receiving adequate mineral nutrition but shaded by *L. italicum*.  $\times \frac{1}{2}$ .
- Fig. 2. Seedlings of *Phleum pratense* 6 months old which have been in competition with *L. italicum*, but over a period of 10 weeks have grown under the following conditions: Row A. Receiving adequate light but mineral nutrition removed by plants of *L. italicum*. Row B. Deprived of both light and mineral nutrition by plants of *L. italicum*. Row C. Receiving adequate light and mineral nutrition. Row D. Receiving adequate mineral nutrition but shaded by *L. italicum*.  $\times \frac{1}{2}$ .

## PLATE XI.

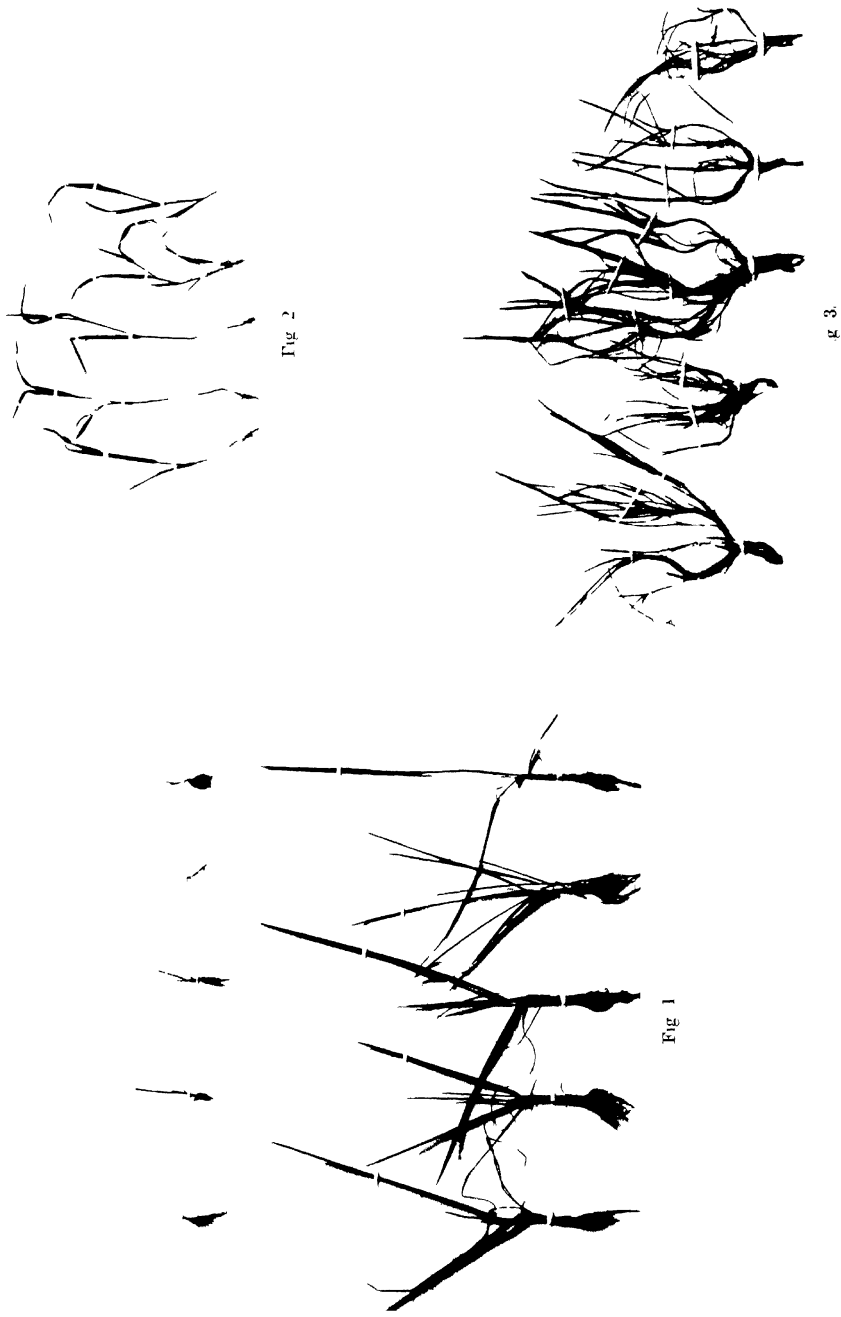
- Fig. 1. Seedlings of *F. pratensis* 10½ months old showing the difference in size between those which have been in competition with seedlings of *L. italicum* older than themselves (upper row), and those which have grown alone (lower row).  $\times \frac{1}{2}$ .
- Fig. 2. Seedlings of *Phleum pratense* 13 weeks old showing the difference in size between those which have been in competition with seedlings of *L. italicum* older than themselves (upper row), and those which have grown alone (lower row).  $\times \frac{1}{6}$ .
- Fig. 3. Seedlings of *Poa trivialis* at least 3 months old, showing the difference in size between those which have been in competition with seedlings of *L. italicum* (upper row), and those which have grown alone (lower row)  $\times \frac{1}{2}$ .

(Received November 25th, 1931.)



CHIPPINDALE.—THE OPERATION OF INTERSPECIFIC COMPETITION IN CAUSING DELAYED GROWTH OF GRASSES (pp. 221-242).





CHIPPINDALE —THE OPERATION OF INTERSPECIFIC COMPETITION IN CAUSING DELAYED GROWTH OF GRASSES (pp. 221-242).





# ON THE GALL MIDGES INJURIOUS TO THE CULTIVATION OF WILLOWS

## I. THE BAT WILLOW GALL MIDGE (*RHABDOPHAGA TERMINALIS* H.LW.)

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(With Plates XII and XIII.)

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### 1. INTRODUCTION.

THE larvae of several species of gall midges (Cecidomyiidae) are responsible for serious damage done to commercially grown willows. They may be conveniently divided into three groups according to the type of injury they cause. Firstly, there are those which destroy the terminal bud or growing point, resulting in the production of side shoots. Secondly, there are those living in the stems or rods. As a consequence rot is liable to develop and this entails in some cases a reduction in the number of sets which normally would be cut from healthy rods, in other cases weak points in the rods when being worked up into baskets, and in others unhealthy stubs. Thirdly, there are those which cause galls to form on the leaves. The presence of this type of damage does more to reduce the value of the standing crop in the eyes of possible buyers than actual injury to the rods, except very occasionally in years of great abundance when the plants suffer from the diminished surface of healthy leaves.

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It is proposed to deal with several of these midges as opportunity arises. The button top midge (*Rhabdophaga heterobia* H.Lw.) has already received attention<sup>1</sup>. In this paper it is intended to give an account of the bionomics of the bat willow gall midge (*Rhabdophaga terminalis* H.Lw.).

I should like to state here how much I am indebted to Mr H. P. Hutchinson, the willow research officer at Long Ashton Research Station, for advice and providing sets of various species of willow; to Dr Imms who has supervised and discussed the work; and to all those whose knowledge of field conditions has been placed at my disposal, especially Mr Roebuck of the Midland Agricultural College, and Mr N. B. Warner Bromley.

### 2. DESCRIPTION OF ADULT AND DISTRIBUTION.

*Rhabdophaga terminalis* was first described by H. Loew (1850) as *Cecidomyia terminalis*. Later writers placed it in the genus *Perrisia* (*Dasyneura*), but now it is usually considered as belonging definitely to the genus *Rhabdophaga* Westw.

*R. terminalis* is typical of this genus, all of whose members bear a very close resemblance to species of the genus *Dasyneura* except that in *Rhabdophaga* the third vein joins the wing margin near or at the apex of the wing. The antennae consist of two basal segments and from 12 to 16 flagellar segments in the male and 11 to 15 in the females. The palps are composed of four segments, the fourth being the longest. The general size of the midge varies considerably, the body length being from about 1 mm. to slightly over 2 mm. In the female the abdomen is bright red until the eggs have been deposited, when it assumes a dark brown appearance similar to that of the male.

Until further studies of allied species have been made, it is not considered possible to identify this species on morphological characters alone, biological ones being essential.

The bat willow midge is recorded from western and central Europe, Denmark, Italy and England. In the last named country it has been found in Kent (Zimmermann, 1907), Wiltshire, and Suffolk and Norfolk. It is probably widely spread throughout the country. While not occurring in every commercial willow bed, in places where it does occur it is very liable to be exceedingly abundant.

<sup>1</sup> Barnes, H. F., "Button Top" of Basket Willows, *Journ. Min. Agric.* April, 1929, pp. 65-71; On the Resistance of Basket Willows to Button Gall Formation, *Ann. Appl. Biol.* xvii, 1930, pp. 638-40; Further results of an investigation into the Resistance of Basket Willows to Button Gall Formation, *loc. cit.* xviii, 1931, pp. 75-82.

## 3. BIONOMICS.

(a) *Emergence, mating, sex ratio and variation in adult.*

Emergence of the adults takes place at a definite time of the day; starting slightly before 8 a.m. (standard time), the maximum emergence of the males occurs about 10 a.m. and that of the females about noon, while very few individuals emerge after 5 p.m. (Barnes, 1930).

The males remain hovering round the soil from which emergence is taking place, awaiting the females. Fertilisation takes place immediately and without ceremony, coition lasting between 30 and 90 secs. One male will impregnate several females. Mating appears to be dependent on some chemotropic factor, as males have been observed hovering furiously round a position, *e.g.* leaf tip, which a virgin female has just left and have taken several minutes to find her in a new position. In the same way a male has been seen attempting to mate with a squashed virgin female when in reality there was only a shapeless mass remaining<sup>1</sup>. Various experiments have been conducted to see whether this species will mate with the button top midge (*R. heterobia*) and the leaf-curling pear midge (*D. pyri* Bouché). Negative results have been obtained in every case, no excitement of the males, no protrusion of the ovipositor by the females and no mating taking place.

The sex ratio at the time of emergence is rather interesting. It has been found previously (Barnes, 1931 *a*), that individuals reared from larvae collected in the field from golden willow (*S. alba* var. *vitellina*) gave a ratio of about 30 : 70, while individuals obtained in a similar manner from adjacent rows of bat willow (*S. coerulea*) gave ratios of 57 : 43, 70 : 30 and 16 : 84. These types of sex ratio were confirmed by breeding experiments under cold greenhouse conditions. Midges were reared in seven pots of *S. alba* var. *vitellina* and the sex ratio of the total midges bred was 16 : 84; on the other hand, using *S. coerulea* as the host plant in a similar number of pots, the figure of 54 : 46 was obtained. A possible reason for this wide range is the occurrence of unisexual progenies. It was found that in certain cases, where only a few females were used in setting up experiments, the subsequent generation consisted entirely of one sex. Allowing for the fact that it is quite possible that some of the females did not oviposit, it is highly probable that this

<sup>1</sup> Occasionally in mating experiments with this species, when several males have been present in the company of virgin females, homosexuality has been observed, but in every case the offending male has subsequently died, probably as a result of some misplacement. This would prevent the possibility of such a tendency becoming inherited.

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indicates the phenomenon of unisexual families. The following figures are given in support of this hypothesis:

Cage A.	7 ♀♀ produced	0 ♂♂, 117 ♀♀
„ B.	$x$ ♀♀ „	0 ♂♂, 63 ♀♀
„ C.	5 ♀♀ „	0 ♂♂, 70 ♀♀
„ D.	25 ♀♀ „	29 ♂♂, 0 ♀♀

It was found previously (Barnes, 1931 b) in another species of gall midge, *Thomasiniana oculiperda* Rüb.s., that this type of result correctly indicated the occurrence of unisexual families.

The adults of the bat willow midge vary considerably in size (Plate XII, figs. 1 and 2) according to the quantity of food available to the larvae (Barnes, 1932). Individuals may be from about 1 mm. to just over 2 mm. in body length. Besides this, the number of antennal segments may be from 2 + 11 in small females to 2 + 15 in large ones and from 2 + 12 in small under-nourished males to 2 + 16 in well-nourished ones.

An unusually coloured female was reared in 1928, the normal red colour of the abdomen being entirely absent and replaced by a whitish colour (Cecid. 1274).

### (b) *Oviposition, types of gall and damage.*

Oviposition takes place a few minutes after mating. The eggs are laid in the terminal buds, being pushed in between the folds of the unopened leaves. Occasionally they are placed at the extreme base of lateral buds or at the base of the petioles. They are bright shining red in colour and the larvae hatch within 8 days or so, according to weather conditions.

The normal type of damage is shown in Plate XII, fig. 3 and Plate XIII, fig. 4. The terminal leaves remain in a curled and crinkled state instead of unfolding naturally. The gall is at first reddish in colour, but when the larvae have finished feeding it turns black and dries up. This holds good whether the pupae are in the gall or not. Occasionally one may find blister-like galls, as shown in Plate XIII, fig. 5, along the mid-vein of the leaf. This type occurs when, owing to adverse weather conditions, the development of the eggs is retarded to a greater degree than the growth of the plant. In such cases the leaves on which the eggs have been laid in the terminal bud develop in a normal manner, but the larvae hatch after a longer period than usual and not until after terminal growth of the plant has left these leaves down the stem, some distance from the growing point. The same type of gall results when the eggs are laid at

the base of lateral buds and petioles. Usually the larvae hatch very quickly and produce the crinkled appearance of the leaves on which they have been laid before any appreciable amount of plant growth has taken place. As soon as the galls start developing terminal growth comes to an end.

Another position where the larvae may be found is in the galls of *Rhabdophaga rosaria* H.Lw., the large rosette-like galls which occur on various species of hedgerow *Salix* as well as on bat willow. Here the larvae live asinquilines. Individuals of the bat willow gall midge have been reared from galls of this species and have been found to interbreed quite normally with those from the usual type of gall. F. Loew (1875) states that the larvae of *R. heterobia* live occasionally as inquiline of *R. rosaria*. But one must now consider an error was made in the identification of *R. heterobia* and that he was dealing with the bat willow midge, *R. terminalis*. Individuals reared from larvae living as inquiline of *R. rosaria* will mate and interbreed with *R. terminalis* on the one hand; but on the other, *R. heterobia* will not mate at all with *R. terminalis*.

The damage done by this midge is very similar to that caused by the button top midge (*R. heterobia*). Terminal growth of the attacked shoots is stopped and side shoots develop. This renders the rods practically useless. In the case of attacks on golden willow (*S. alba* var. *vitellina*) the rods are spoilt from the basket-making point of view. In the case of attacks on bat willow (*S. coerulea*) it is much more serious. The bat willow is grown for cricket bats and it is usual to plant sets of 8-12 ft. long and slightly thicker than broomsticks. Once these sets are planted they should not be moved, and the trees should be clear of branches at least 15 ft. from the ground. A method of growing these sets is to allow rods from a stub to continue growing for a period of 2-3 years before cutting and then transplant the sets into their permanent positions. It is absolutely essential that the sets be free from side shoots. The bat willow midge sometimes causes great havoc in places where these sets are being grown and owing to their value the monetary loss is very considerable.

The bat willow midges living as inquiline in the galls of *R. rosaria* cannot be considered to be directly injurious, as the larvae of *R. rosaria* are the cause of the side branching in these cases. But, since *R. rosaria* occurs on indigenous hedgerow species of *Salix*, the presence of the inquiline is an additional reservoir of infection which can easily be overlooked.

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### (c) *Host plants with results of preference and immunity trials.*

Previous workers have recorded this midge from several species of *Salix*, including *S. alba*, *fragilis*, *amygdalina*, *hastata*, *pentandra*, *purpurea*, *viridis* and *repens* (Houard, 1908; Kieffer, 1901). Such records must be considered unproven until biological work has shown that this midge will attack such a wide range of *Salix* species.

Investigations carried out at Rothamsted have shown that there are definite host plant preferences and immunities. The experiments were carried out in the same manner as those described in the trials of the resistance of basket willows to the button top midge (Barnes, 1931 c).

In the field this species has only been found attacking the bat willow (*S. coerulea*) and a golden willow, *S. alba* var. *vitellina*.

Midges, when given a choice of ovipositing on bat willow (*S. coerulea*) or *S. alba* var. *vitellina*, invariably chose the bat willow<sup>1</sup>. This was true whether the midges had been reared on *S. coerulea* or *S. alba* var. *vitellina* for one or two generations previously. There was no inclination for those reared on the latter to oviposit on *S. alba* var. *vitellina*. Perhaps if they had been reared for many generations on the one host plant they might prefer to lay on it rather than on *S. coerulea*.

If the midges had no choice but had to lay on *S. alba* var. *vitellina*, they did so quite readily and subsequently the normal development took place.

The following varieties and species withstood all attacks, both in preference and immunity trials: Black Maul (*S. triandra*), Long Skin (*S. viminalis*) and Dicky Meadow (*S. purpurea*).

*S. alba* var. *cardinalis* was subjected to preference trials only, and proved to be immune in the presence of bat willow (*S. coerulea*), *S. alba* var. *vitellina* and Black Maul (*S. triandra*). This does not prove that it is immune in reality, since *vitellina* was not attacked in these circumstances. But it does show that it is not more attractive for oviposition than *S. coerulea*.

### (d) *Life cycle.*

The galls of the last brood of the year fall to the ground in the autumn with the leaves, carrying with them any larvae that have not previously dropped to the soil. The larvae remain in the surface layers

<sup>1</sup> For example, in one instance, 1931, in a cage containing 9 bat willow and 10 *vitellina* plants, 8 out of the 9 bat plants were attacked but none of the *vitellina* ones. As the plants were randomised, the ovipositing females must have flown up and down the cage choosing the bat plants in distinct preference to the *vitellina*.

until the following spring. Pupation of this generation takes place in the soil.

In early May the first flight of the adults takes place, and subsequently further generations are on the wing at about monthly intervals throughout the summer until September. Each flight, except the first which may go on for as long as a month, usually lasts about a week or 10 days. The larvae of the summer broods pupate in the galls as a rule but can do so equally well in the ground if they are disturbed or knocked out of the gall either by birds or overcrowding. Up to 40 larvae have on occasion been found in a single gall. The duration of the pupal stage is about a week.

Under unheated greenhouse conditions in 1931 four generations were completed during the summer, thus there were five flights during the year. Only partial emergences of the third and fourth generation, and none of the fifth, took place the same year. The actual dates of oviposition and emergence, together with the duration of the generation can be seen in Table I.

Table I.

*Showing dates of oviposition, flights of adults and time taken to complete the life cycle under unheated greenhouse conditions, 1931.*

Date of oviposition	Dates of flight of midges	Time in days taken to complete life cycle
May 10-11	June 13-19	34
" 12	" 13-15	32
" 13	" 13-19	31
June 13	July 6-19	23
" 14	" 6-11	22
" 13-14	" 9-12	26
" 13-14	" 8-18	25
July 6	Aug. 1-6	26
" 6-11	" 1-6	26
" 7-8	" 2-9	26
" 8	" 2-11	25
" 7	" 5-16	29
" 7	" 4	28
Aug. 4-5	Sept. 1-10	28
" 2-5	Aug. 30-Sept. 10	28
" 3-4	" 31- " 10	28

The midges were reared both on bat and golden willow. It is interesting to note that the cycle took 31-34 days to complete in May-June, but less (22-26) in June-July, and slightly longer again in July-August (25-29) and August-September (28). In a previous year in the same greenhouse there was one flight less.



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Observations in the field suggest that there are usually four flights during the year, that in more favourable seasons it is probable there is at least one more and in unfavourable ones probably one less. Considerable overlapping takes place, however, it being possible to find adults and larvae in all stages almost at any time between late May and the end of August.

It is also considered as highly probable, if not certain, that some individuals of the antipenultimate and penultimate generations of the year remain in the soil as larvae, not emerging until the following spring. It has been proved that they do this when reared in pots and under unheated greenhouse conditions, and also that fully fed larvae of the penultimate generation of the season obtained from the field and kept in pots in an outdoor insectary do likewise.

### 4. CONTROL.

There is no real control yet known for this midge. Certain Hymenopterous Chalcid parasites are usually present but do not appear to breed fast enough to exert any very great control. The bug *Anthocoris nemorum* L. is frequently found sucking either adults as they are emerging or the larvae in the galls. Tits also eat considerable numbers of the larvae, picking them out of the galls. In spite of these natural enemies, this midge seems to multiply very rapidly, and by the late summer in some years there must be literally millions in some willow beds. Fortunately this midge, although liable to appear in epidemic proportions on occasion, is often noteworthy because of its greatly diminished numbers. Some factor or factors in the weather complex seem to have a controlling influence over it. What this is still remains to be discovered; it may have a direct effect on the midge itself or it may have differential effects on the host insect and its parasites and so be acting indirectly on the midge.

As to artificial control measures there is unfortunately little to say. Handpicking the galls can be suggested where school children can be employed cheaply. It is suggested that cultivating the soil between the stubs very thoroughly in March and April might diminish the numbers of the first flight in May very considerably. Massee (1931) has reported that he found cultivation very effective in the case of the pear midge (*Contarinia pyrivora*). The essential feature is that the cultivator must be taken across the rows, along the rows and also diagonally across them. It should be done once a week for about 4 or 5 weeks with a view to exposing the larvae. Hand hoeing round the stubs must accompany

any such cultivation. In the case of the pear midge the cultivation was done immediately the larvae had descended to the soil about June 10th, but this midge is only single brooded. The cultivation in March and April is recommended in the case of the bat willow midge because it is about this time that all the larvae are in the soil. Throughout the summer there are larvae up in the galls. It might be effective to do the cultivation in the late autumn if it were a rather cold one. But the east winds, general lack of insect food for birds, and the possibility of frosty weather in March make this period seem more advantageous. The cost of such extensive cultivation would be amply compensated for by the value of clean cricket bat sets; in the case of golden willows it might not be economical.

All hedgerow *Salix* bushes should be exterminated, as they may be acting as reservoirs of this midge ready to re-infest the fields.

#### 5. SUMMARY.

1. The bionomics of the bat willow gall midge (*Rhabdophaga terminalis* H.Lw.), which does serious damage to certain willows grown for basket making and the cricket bat willow grown for sets, have been studied.

2. The midge exhibits a distinct host-plant preference, choosing the bat willow (*S. coerulea*) when possible. But it also breeds readily on a golden willow, *S. alba* var. *vitellina*. It will not attack Black Maul (*S. triandra*), Long Skin (*S. viminalis*) and Dicky Meadow (*S. purpurea*).

3. It is shown to be a species which sometimes occurs in epidemic numbers. Intensive cultivation is suggested as a control.

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### EXPLANATION OF PLATES XII, XIII.

#### PLATE XII.

- Fig. 1. Small female *R. terminalis*,  $\times 20$ .
- Fig. 2. Large female *R. terminalis*,  $\times 15$ .
- Fig. 3. Typical gall of *R. terminalis* in dried up condition denoting the presence of pupae, showing damage by *Phyllodecta vitellinae* on the outer leaves.

#### PLATE XIII.

- Fig. 4. Four typical galls containing larvae on *S. alba* var. *vitellina*.
- Fig. 5. Alternate type of gall, when the terminal bud has grown away from the attack, or when the eggs have been laid at the base of lateral buds and petioles instead of on the terminal bud.

(Received November 25th, 1931.)

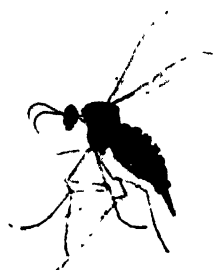


Fig 1



Fig 2



Fig 3.





Fig. 4.



Fig. 5.



# THE INSECTICIDAL PROPERTIES OF *TEPHROSIA MACROPODA* HARV. AND OTHER TROPICAL PLANTS

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DURING the past few years a considerable number of plants received from different parts of the Empire and elsewhere have been examined for their toxicity to insects. Most of these plants belong to the order Leguminosae; they come chiefly from tropical countries and many of them are known to be employed by the natives of the districts where they occur as "fish poisons." A few plants belonging to other natural orders and some which are not fish poisons have been included.

The best known of these fish-poison plants is *Derris* or tuba-root, which is already widely used as an insecticide; others possessing high insecticidal powers and possibly of commercial importance are *Tephrosia vogellii* Hooker, *T. toxicaria* Pers. and species of *Lonchocarpus*<sup>1</sup> known as black and white Haiari. These have been the subjects of separate investigations, the results of which have already been published (1).

Among numerous other plants examined, the great majority proved to be without insecticidal properties or to possess them only in a slight degree, but a few were definitely toxic to insects, and may be worth further investigation. It is thought that the data accumulated should be put on record, although detailed studies of these plants have not at present been attempted.

In making preliminary trials, the plant material was ground finely, extracted with alcohol or water, and the extracts diluted with a 0.5 per cent. solution of non-toxic saponin. A high concentration (equivalent to 1-5 per cent. of the plant material) was tried in the first place. Laboratory spraying tests were then carried out with these extracts by means of the apparatus and method which have been already described (2). The insect used in most of the experiments was the Black Bean Aphid

<sup>1</sup> Another species of *Lonchocarpus*, known as Cubé, has also been found to be very toxic to insects and has been studied in America.



## 254 *Insecticidal Properties of Tephrosia macropoda Harv.*

(*Aphis rumicis* L.), feeding on Broad Bean plants, and bred as far as possible under standardised conditions.

The following is a list of plants<sup>1</sup> samples of which showed little or no toxicity to *Aphis rumicis* when tested under these conditions.

Species	Natural order	Derivation	Parts tested
* <i>Albizia stipulata</i> Boiv.	Leguminosae	India	Leaves and bark
* <i>A. procera</i> Benth.	"	"	"
* <i>Acacia pruinescens</i> Kurz.	"	Burma	"
* <i>A. salicina</i> Lindl.	"	Australia	" (2 specs.)
<i>A. falciformis</i> D.C.	"	"	"
* <i>A. pennata</i> Willd.	"	Burma	"
<i>Caconia coccinea</i>	Combretaceae	British Guiana	Shell and kernel of fruit
*† <i>Cassia didymobotria</i> Fres.	Leguminosae	Kenya	Roots, stems, leaves, seed
* <i>C. hirsuta</i> L.	"	Malaya	"
* <i>Clibadium vargasii</i> (known as Nivrai)	Compositae	Antigua	Leaves, stems
† <i>Clitoria macrophylla</i> Wall.	Leguminosae	Siam	Roots
<i>Derris scandens</i>	"	British Guiana	Roots, stems, leaves
* <i>D. trifoliata</i> (uliginosa)	"	India, Siam ✓	Branches, roots, leaves
* <i>Dolichos lupiniflorus</i>	"	Southern Rhodesia	Roots
<i>Drepanocarpus lunatus</i>	"	British Guiana	Leaves, stems, roots, fruit
* <i>Euphorbia hiberna</i> L.	Euphorbiaceae	Ireland	Stems, leaves
* <i>E. cotinoides</i> (known as Conaparu)	"	British Guiana	"
<i>Haronga paniculata</i> Lodd.	Guttiferae	Sierra Leone	Bark
<i>Jaquinia ruscifolia</i>	Theophrastaceae	British Guiana	Stems, leaves
* <i>Lonchocarpus latifolius</i> Pers.	Leguminosae	Trinidad	Seeds, pods
*†	"	—	"
† <i>Mammea americana</i> L.	Guttiferae	Trinidad	Roots, shoots, branches
† <i>Melia azadirachta</i> L.	Meliaceae	India ✓	Leaves
* <i>Milletia pachycarpa</i> Benth.	Leguminosae	Burma	Bark
*† <i>Neurolaena lobata</i> (known as Erb-à-picque)	Compositae	Antigua	Leaves, stems
* <i>Ougeinia dalbergioides</i> Benth.	Leguminosae	India ✓	Leaves, bark
<i>Chrysanthemum</i> ( <i>Pyrethrum</i> ) <i>frutescens</i>	Compositae	Uganda	"
* <i>Phyllanthus conami</i> Sw. (known as Danconami or Daukanani)	Euphorbiaceae	British Guiana	Roots, stems, leaves
*† <i>Pithecolobium elliptica</i> Hassk.	Leguminosae	Malaya	Leaves, bark
<i>Physalis angulata</i>	Solanaceae	British Guiana	Whole of plant
<i>Pulicaria dysenterica</i> Gaertn.	Compositae	England	Leaves, flowers, stems, roots
<i>Stemona collinsae</i> Craib.	Stemonaceae	Siam	Tubers
*† <i>Tephrosia candida</i>	Leguminosae	Trinidad	Stems, roots
* <i>T. purpurea</i> L.	"	India?	Roots, stems, leaves
* <i>T. heckmanniana</i>	"	Southern Rhodesia	Stems and leaves together
*† <i>T. hookeriana</i>	"	" ✓	Roots, stems, leaves, fruits
<i>Uvaria latifolia</i> Prain	Anonaceae	Siam	Roots
*† <i>Conami clibadium</i> (Clibadium ? Surinamense)	Compositae	British Guiana	Roots, stems, leaves, flowers, fruit
*† <i>Hebitchoahabu</i> ( <i>Serjania</i> sp.)	Sapindaceae	"	Stems
*† <i>Moroballi</i> ( <i>Muraballi</i> ) ( <i>Cupania</i> sp.?)	Sapindaceae?	"	Wood, bark

The precise identity of the last three plants is doubtful. Plants known to be fish poisons are marked \*. Plants showing some slight toxicity are marked †.

<sup>1</sup> All plants from abroad were received in dry condition. It is possible that infusions of fresh leaves or stems might in some cases give different results.

The preliminary experiments with these plants having given negative, or almost negative results, no further time was spent on them. It is possible, however, that *Lonchocarpus latifolius* would justify further examination; the seeds and pods showed some slight toxicity and, in view of the powerful insecticidal properties possessed by certain other members of the genus, it would be of interest to test the leaves, stems and roots of this plant. The seeds of *Tephrosia hookeriana* also showed some toxicity.

Aqueous infusions of the fresh leaves of *Tephrosia heckmannia* were reported by Mr H. C. Arnold of Southern Rhodesia *in litt.* to have toxicity to bed bugs and to larvae of the maize stalk borer; but extracts of the dry material received were harmless to *Aphis rumicis*.

The following plants showed definite activity as contact insecticides: *Mundulea suberosa* Benth. from India; *Neorautanenia fisifolia* (Benth.) C.A.Sm. (= *Rhynchosia fisifolia* Benth.) from Southern Rhodesia; *Tephrosia macropoda* Harv. from Natal. Further experiments were therefore made with these species, and are reported here.

#### *TEPHROSIA MACROPODA* Harv.

The genus *Tephrosia* of the Leguminosae includes several species utilised in many parts of the world as fish poisons and, as already mentioned, some are also insecticidal. *T. macropoda* is the best known fish-poison plant in South Africa, and goes by the native name of "ilozane." The following particulars with regard to this plant are given by Howes (3) in a paper on fish-poison plants:

"It is common in the coastal grassveld of Natal and extends irregularly over the greater part of South-East Africa. The plant, which is of a more or less trailing habit, is characterised by a somewhat fleshy variously shaped rootstock. It is this portion of the plant that is used, being merely mashed between stones at the side of a pool or stream before use by the Zulus and other tribes. As legislation now exists against its use in Natal it is not employed as much as it was formerly. An infusion of the roots with water was commonly used by settlers in the early days in Natal as a wash for freeing dogs of fleas and ticks."

Mr W. V. Blewett kindly forwarded samples of *T. macropoda* from Natal some years ago and a note of some preliminary experiments with extracts of the roots and stems as stomach poisons has been published (4). A very strong repellent action to young larvae of the winter moth (*Cheimatobia brunata* L.) was observed.

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Experiments were made on the action of alcohol extracts of various parts of the plant as contact insecticides in 1926 and 1927, using the black bean aphid, *Aphis rumicis*, under the conditions to which reference has been made. Similar contact experiments were also carried out with larvae of the moths *Selenia tetralunaria* and *Orgyia antiqua* L., the same apparatus and general technique being adopted as with *Aphis rumicis*. After spraying, each lot of ten larvae were immediately transferred to a separate cage with ample fresh food plant, and kept under observation for several days. The data obtained in both sets of tests are set out in Tables I and II.

Table I.

### *Toxicity of alcohol extracts of Tephrosia macropoda Harv. to Aphis rumicis L.*

[N=not affected; S=slightly affected; M=moribund; D=dead.]

Date	Material	Concentration: % of plant material	N %	S %	M %	D %	M and D %
1926							
July 29	Roots and stems	1.0	—	—	10	90	100
		0.25	—	—	20	80	100
	Control (0.5 % saponin)	—	95	—	5	—	5
Aug. 25	Leaves	1.0	50	10	20	20	40
		0.2	90	—	—	10	10
	Stems	1.0	—	—	20	80	100
		0.2	30	30	20	20	40
	Control (0.5 % saponin)	—	100	—	—	—	0
Aug. 28	Roots	0.5	—	—	90	10	100
		0.25	10	—	50	40	90
		0.1	20	10	70	—	70
		0.05	50	20	30	—	30
		0.025	90	—	10	—	10
	Stems	1.0	—	—	50	50	100
		0.75	—	—	50	50	100
		0.5	—	—	60	40	100
		0.25	—	30	40	30	70
		0.1	—	50	50	—	50
	Controls (0.5 % saponin and 5-10 % alcohol)	—	100	—	—	—	0
1927							
June 9	Roots and stems	0.5	—	—	40	60	100
		0.25	—	—	60	40	100
		0.1	20	30	30	20	50
June 24	Roots: very roughly ground	0.5	—	—	80	20	100
		0.25	—	10	70	20	90
		0.1	80	20	—	—	0
	Stems: very roughly ground	0.5	100	—	—	—	0
		0.25	100	—	—	—	0
		0.1	80	20	—	—	0
	Control (0.5 % saponin)	—	80	10	5	5	10

The counts were made two days after spraying.

Table II.

*Toxicity of alcohol extracts of Tephrosia macropoda Harv. to larvae of Selenia tetralunaria Hufn. and Orgyia antiqua L.*

[N=not affected; A=affected; M=moribund; D=dead.]

Material	Concentration: % of plant material	Larvae	N	A	M	D	Feeding and growth
Roots (old extract)	1.0	<i>S. tetralunaria</i> (young)	—	—	1	9	None
	0.5	—	—	2	—	7	Very slight
Control (0.25 % soap solution)	—	—	10	—	—	—	Normal
Control (unsprayed)	—	—	20	—	—	—	Normal
Roots (old extract)	1.0	<i>S. tetralunaria</i> (about $\frac{1}{2}$ grown)	—	3	2	5	Slight
	0.5	—	—	7	—	3	Appreciable
Roots (fresh extract)	1.0	—	—	3	6	1	Very slight
	0.5	—	—	9	—	1	Considerable
Control (0.25 % soap + 20 % alcohol)	—	—	20	—	—	—	Normal
Control (unsprayed)	—	—	20	—	—	—	Normal
Roots (fresh extract)	1.0	<i>O. antiqua</i> (about 1 month old)	—	3	—	6	Very slight
	0.5	—	—	2	1	7	Very slight
	0.25	—	—	4	—	6	Appreciable
Controls (0.25 % soap solution)	—	—	18	—	—	1	Normal
Controls (unsprayed)	—	—	20	—	—	—	Normal

Final counts made 7 days after spraying. Larvae in column A were in a semi-paralysed condition and it is improbable that they would have completed development. Those in column D were killed almost immediately by the spray.

It is apparent that alcohol extracts of the roots, and, to a less extent, of the stems of *Tephrosia macropoda* possess considerable contact insecticidal properties. The leaves, on the other hand, are of little value, from this point of view. There is some discrepancy between the figures obtained at different dates (see Table I), but this is probably accounted for by less complete extraction of the active principles in the 1927 experiments when the samples were only roughly ground. Moreover, it was not found very easy to separate root and stem exactly, and some of the toxic effect of the stems in the earlier tests may have been due to the inclusion of a proportion of roots. The roots are undoubtedly very toxic and warrant further consideration as a possible insecticide. The plant is apparently common in certain areas in Natal and could presumably be cultivated without great difficulty.

*MUNDULEA SUBEROSA BENTH.*

This is a common leguminous plant occurring in most parts of tropical and sub-tropical Africa, in Madagascar, and in India and Ceylon. It has long been cultivated and the seeds and bark used as fish poisons (see Howes, *loc. cit.*). It is said to be very rapid and potent in its action on fish. Roark<sup>(5)</sup> reports that the seeds and inner layer of bark are used both as fish poisons and insecticides in India; and he includes this species<sup>(6)</sup> in a list of plants, other than Derris, which contain rotenone.

Two samples of *M. suberosa* were received, and differed very markedly in the effect of their extracts on *Aphis rumicis*. Specimens from South Africa sent by the Bureau of Plant Industry, Pretoria, which included stems, bark, cork and leaves, proved to have no appreciable toxicity. On the other hand, the stems, seeds and pods of another sample, from India, forwarded by the Divisional Forest Officer, Dharwar, Bijapur, were quite toxic as the figures in Table III show; the roots and leaves however had no appreciable action at a concentration equivalent to 1 per cent. of the plant material. The stems were the most active part of the plant. The action on the aphides was somewhat delayed; it began with partial paralysis which gradually deepened until the insects became moribund and finally died. The counts given were taken 3 days after spraying.

Table III.

*Toxicity of alcoholic extracts of stems, seeds, and pods of  
Mundulea suberosa Benth. to Aphis rumicis L.*

Concentration: % of plant material	% insects moribund and dead
1.0	100
0.5	100
0.25	20

These figures, while showing the plant to be toxic, do not suggest that it would take a very high place among fish-poison plants as an insecticide. Nevertheless, if, as seems to be the case, it is easy to grow, and if harvesting of the valuable parts of the plant does not present difficulties, the cultivation of *Mundulea suberosa* might prove to be an economic proposition in certain tropical areas.

*NEORAUTANENIA FISIFOLIA* (BENTH.) C.A.SM. (= *RYNCHOSIA FISIFOLIA* BENTH.).

This is another leguminous plant which occurs in various parts of eastern South Africa, particularly the Transvaal and Natal, and in Southern Rhodesia. Specimens of the large tuberous roots were received from Mr H. C. Arnold of the Division of Plant Industry, Department of Agriculture of Southern Rhodesia, who states that the natives use them, when mixed with the roots of another legume, *Dolichos lupiniflorus*<sup>1</sup>, as fish poisons. He writes: "The two kinds of roots are thoroughly pounded and mixed, mud is added and the mixture thrown into the pool of water which is vigorously stirred. After an hour or two the fish rise to the surface in an intoxicated condition and many of them die within 24 hours." Mr Arnold also reports that an infusion prepared by soaking the powdered root in water for a few days was found to kill bed-bugs and the larvae of the maize stalk borer (*Busseola fusca* Fuller) after momentary immersion. The infusion was harmless to the foliage of maize plants.

Both alcoholic and aqueous extracts of the ground roots diluted with 0.5 per cent. saponin solution were tested and proved to be toxic to *A. rumicis*. Table IV shows the results obtained with the alcohol extract. Control experiments with 0.5 per cent. solutions of saponin containing 1.0, 2.5, 5.0 and 10 per cent. of alcohol showed an average mortality of 10 per cent. of the insects sprayed (maximum, in one test only, 20 per cent.).

Table IV.

*Toxicity of alcoholic extracts of tuberous roots of Neorautanenia fisifolia C.A.Sm. to Aphis rumicis L.*

Concentration: % of plant material	% insects moribund and dead
1.0	100
0.5	80
0.25	70
0.1	20

The toxic action of these extracts (as with those of *Mundulea suberosa*) was rather delayed, insects only slightly affected at certain concentrations gradually sinking into a moribund state 12 or 15 hours after spraying. The final counts were taken after 3 days.

It is evident from these figures that the roots of *Neorautanenia fisifolia* possess quite considerable insecticidal properties and would be worth further study with a view to use locally where the plant is readily obtainable.

<sup>1</sup> The roots of *D. lupiniflorus* were also examined but showed no toxicity to *A. rumicis*: see list on p. 254.

BLACK HAIARI (*LONCHOCARPUS* SP.).

Data have been previously published with regard to the high toxicity to *Aphis rumicis* of extracts of the stems of black Haiari (*Lonchocarpus* sp.) as contact insecticides (1), and to their strongly repellent action to the larvae of several species of moths, when tested as stomach insecticides (4). As supplementary to these experiments, the results of a small number of tests on the contact insecticidal action of these extracts on caterpillars are of interest and are recorded in Table V. The method adopted for these tests has been referred to in the section dealing with *Tephrosia macropoda*.

Table V.

*Toxicity of alcoholic extracts of black Haiari to larvae of Selenia tetralunaria Hufn. and Orgyia antiqua L.*

[N = not affected; A = affected; M = moribund; D = dead.]

Material		Concentration: % of plant material	Larvae	N	A	M	D	Feeding and growth
Black Haiari stems (old extract)		1.0	<i>S. tetralunaria</i> (young)	—	4	1	4	Slight
		0.5	—	—	8	—	2	Slight
Black Haiari stems (fresh extract)		1.0	—	—	—	—	10	None
		0.5	—	—	—	—	10	None
Control (0.25 % soap solution)		—	—	10	—	—	—	Normal
Control (unsprayed)		—	—	20	—	—	—	Normal
Black Haiari stems (extract 14 days old)		1.0	<i>S. tetralunaria</i> (about $\frac{1}{2}$ grown)	9	—	—	—	Considerable
		0.5	—	10	—	—	—	Almost normal
		0.25	—	10	—	—	—	Normal
Black Haiari stems (fresh extract)		0.5	—	—	7	—	2	Appreciable
		0.25	—	—	10	—	—	Considerable
Control (0.25 % soap solution and 20 % alcohol)		—	—	20	—	—	—	Normal
Control (unsprayed)		—	—	20	—	—	—	Normal
Black Haiari stems		1.0	<i>O. antiqua</i> (about 1 month old)	4	2	1	3	Considerable
		0.5	—	1	4	—	5	Considerable
		0.25	—	2	3	1	4	Considerable
Control (0.25 % soap solution)		—	—	18	—	—	1	Normal
Control (unsprayed)		—	—	20	—	—	—	Normal

Final counts made 7 days after spraying.

It will be seen from Table V, that extracts of this plant are toxic as contact insecticides to larvae of the two species used<sup>1</sup>. The toxicity of the extracts is indeed somewhat greater than is apparent, for the insects included in column A in the table were in all cases partially paralysed and capable of but little feeding; it is very unlikely that any of these would have completed development. Insects in column D were all killed almost immediately by the spray. The figures also indicate that older larvae are much more resistant than young ones to the effect of the extracts and suggest the importance of early spraying if plant insecticides of this type are used against caterpillars. It appears further that alcoholic extracts may lose a proportion of their toxicity if kept for some months.

#### CONCLUSIONS.

The experiments with fish-poison plants which have been described indicate that although some of these plants have a relatively powerful insecticidal action, such properties are by no means always correlated with a stupefying or poisonous action upon fish. Nor is this to be expected, since compounds such as tannins, saponin and even sugars may be lethal to fish but are in general harmless to insects. Time has not been available for any attempt to separate the active principles from those plants which proved toxic to insects, but it seems very probable that they belong to the class of substances of which rotenone is the characteristic and best known member.

It is interesting that all the plants so far examined which possess both insecticidal and fish-stupefying properties belong to the natural order Leguminosae and a more complete survey of plants of this order may be expected to bring further examples to light. The data recorded here and in previous papers are perhaps sufficient to indicate that there is a wide field of work, likely to produce results of economic importance, in the search for possible new insecticidal plants and in the more detailed investigation of the range of activity and usefulness of those already known.

#### SUMMARY.

1. Preliminary data is reported as to the insecticidal properties of three tropical fish-poison plants (*Tephrosia macropoda* Harv., *Mundulea suberosa* Benth. and *Neorautanenia (Rhynchosia) fisifolia* C.A.Sm.).
2. A list is given of other plants (most of them known to be fish

<sup>1</sup> A single experiment with an old extract of black Haiari stems, using young larvae of *Taeniocampa gothica* L., a Noctuid moth, indicated that this species is highly resistant.



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poisons) from many different countries, which have been tested but appear to have little or no toxicity to *Aphis rumicis* L.

3. Extracts of the stems of black Haiari (*Lonchocarpus* sp.) are shown to be toxic as contact insecticides to young larvae of two species of moths. Older larvae are much more resistant.

4. All the plants so far tested which are toxic both to fish and to insects are members of the natural order Leguminosae.

Sincere thanks are due to the agricultural and botanical officers in many different parts of the Empire who have been to much trouble to collect and forward a large number of interesting plants. The authors also desire to express their indebtedness to the Director and other members of the staff of the Royal Botanic Gardens, Kew, through whom much of the material was received, and who kindly supplied much valuable information about individual species of plants.

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(Received December 2nd, 1931.)

## PROCEEDINGS OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS. I

ORDINARY MEETING held on Friday, December 4th, 1931, at 2.30 p.m. in the Botanical Lecture Theatre of the Imperial College of Science and Technology, London. The Chair was taken by the President, Dr A. D. IMMS, F.R.S.

The programme consisted of a discussion on:

“Laboratory methods as a means of testing the value of chemical substances for the control of injurious Fungi.”

The following papers were read:

- I. Laboratory Examination of Fungicidal Dusts and Sprays. By HUBERT MARTIN, M.Sc., A.R.C.S., F.I.C.
- II. Laboratory Methods for Testing Wood Preservatives. By W. P. K. FINDLAY, B.Sc., A.R.C.S.

### THE LABORATORY EXAMINATION OF FUNGICIDAL DUSTS AND SPRAYS.

By HUBERT MARTIN, M.Sc., A.R.C.S., F.I.C.

(*Research Department, South-Eastern Agricultural College, Wye.*)

THERE are many shades of meaning to the term “laboratory method” when applied to the examination of sprays and dusts applied to plant foliage for the control of fungus diseases. For the purpose of this discussion I think we should adopt the widest definition of the expression. In field trials fungicides are tested or compared under the maximum variation in conditions, and the trial should be repeated for several years in order to obtain a sufficient variation in those factors which influence the behaviour of the fungus, the response of the host plant or the properties of the fungicide. In the laboratory trial the influence of variation in one or more of these factors is eliminated. The value of the laboratory trial rests mainly in the simplification thus effected, without which the investigation of fungicidal efficiency would be difficult and, in some cases, impossible. It follows also that by making constant factors which in the field are variable, the standard error is reduced and results of statistical significance are obtained with fewer replications than are required in the field. The applicability of the results of the laboratory trial will, in general, be governed by the importance of the missing variables. The extent to which the laboratory trial will be confirmed by field trial will be controlled by the correctness of the allowances made for the influence of the missing factors in the interpretation of the results.

The problems which have been attacked by the method of laboratory trial may be divided into two groups, those which concern the direct fungicides which are used against ectophytic fungi, and those which concern fungicides which serve to protect the plant from fungus attack.

#### *Direct fungicides.*

The direct fungicide consists generally of a toxic chemical and ingredients which serve to increase the efficiency of that chemical. The spray, for example, must be able to make intimate contact with the fungus it is to kill, a property often conferred by the addition to the spray of capillary active material such as soaps. A dust may contain ingredients to render the active fungicide more toxic, as lime casein is considered to act in dry-mix sulphur lime. Investigation upon the efficiency of direct fungicides is therefore mainly concerned with toxicity, whether it be the discovery of new toxic materials, the elucidation of the mechanism of toxic action or the influence upon toxic action of the presence of these other ingredients of the spray or dust. As direct fungicides are applied to the fungus in active parasitic growth, the spray must, unless assumptions be made of the nature of toxic action, be applied to the growing fungus. But direct fungicides are used mainly for the control of Erysiphaceae and, as no means have yet been found for the cultivation of these fungi on artificial media, the fungicide must be applied to the infected plant. As its action will be determined by the biological state of the fungus, the main function of the laboratory trial is to eliminate variations on the part of the fungus which influence its reaction to the toxic chemical. The method of biological standardisation developed by Salmon (6) with the hop powdery mildew achieves this purpose. By using plants vegetatively propagated from one parent plant susceptible to the mildew, and by the selection for treatment of leaves and mildew patches in the same stage of active growth, the results of the application of a standard spray are made uniform. Variance due to differences in fungus and host plant is eliminated and the results are generally so clear-cut and definite that statistical treatment is unnecessary. Although there is now some evidence that the effect of a standard spray may, at the beginning of the season, be slightly different to that at the end of the season, it has even been found possible to dispense with a standard spray as a means of co-ordinating trials carried out at different times. As changes in external conditions have so small an effect on the action of the fungicide, it may be concluded that the modification from field conditions to conditions in the unheated greenhouse in which the trials are made is of little importance. The main departure from field conditions is the elimination of variation in fungus and host plant. In the interpretation of the results we must therefore remember that the figures expressing toxicity have relative and not absolute value. Another possible source of discrepancy between its results and those of field trials is introduced by the fact that in the field any protective action on the part of the fungicide comes into play. It has not yet been shown to what extent it is necessary that a direct fungicide should possess protective fungicidal powers.

#### *Protective fungicides.*

The function of the protective fungicide is to prevent establishment of fungus infection. This is usually accomplished by placing on the healthy foliage an adherent chemical from which is formed a substance toxic to the fungus spore. Toxicity is therefore not the sum total requirement in a good protective fungicide and its

efficiency will be dependent on factors which affect the adherence and the appearance or formation of the toxic substance.

*Toxicity of the active fungicide.* This toxic derivative of the protective fungicide has to act on the fungus spore or during the interval between germination and penetration into the plant tissue. For laboratory tests we may therefore use spores, and hanging drop or other types of *in vitro* experiments become possible. The purpose of the laboratory trial is to standardise the spore and to eliminate variation of those factors which affect its germination. The physiological factors influencing germination of fungus spores have been widely studied and the importance of temperature, oxygen relationships which in turn are affected by factors such as size of drop and spore concentration, the biological state of the spore which is conditioned by the nature of the culture medium on which the fungus is grown and the age of the culture, has been shown. Rigorous elimination of variations in these factors does not appear, however, to have achieved marked success in securing uniformity of germination or of germ-tube growth. Wilcoxon and McCallan (23), for instance, examined the variability of germination of a number of fungi by making 250 duplicate tests. With an average figure of 70 per cent. germination, only differences greater than 18.50 per cent. were found to be significant; with an average of 50 per cent. germination only

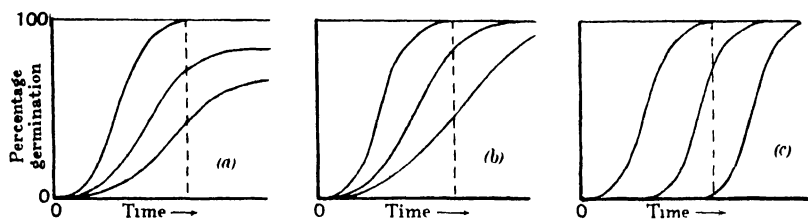


Fig. 1.

percentage germination figures which differed by more than 24.48 per cent. had significance of odds greater than a fifty-to-one-against chance. Such a result is in marked contrast to the success which Salmon obtained in securing uniformity in the biological condition of the hop powdery mildew.

Nor is it a simple matter in spore-germination tests to deduce a satisfactory figure to express toxicity. Unless supported by other data, the figure for percentage germination or for germ-tube length may be misleading. Attention was drawn to this pitfall by Tomkins, in a paper read before the British Mycological Society at the November meeting. The curve of percentage germination against time is generally of the form (a) or (b) in Fig. 1, and it will be seen that the position chosen for the ordinate at which to compare the figures for percentage germination may affect the result. This is especially so in case (c), examples of which were given by Tomkins, and it is obvious that adequate measures must be taken to ensure that a mere retardation of germination is not accepted as non-germination.

This difficulty is avoided in a method of examining the toxic derivative which I have found useful in my work at Wye and which depends upon the cessation of movement and the disintegration of zoospores as a measure of toxicity. The method originated from some observations by Ware (10) on the behaviour of zoospores towards toxic material. To test the action of a sulphur-soft soap suspension as a

substitute for copper-containing sprays, Ware added a suspension of zoospores to the mixture. But in the subsequent microscopic examination he was unable to find the zoospores. The total disappearance of the fungus, Ware found, was due to the action of the soap which, at the concentration used, caused a rapid disintegration or bursting of the zoospore. This disintegration is preceded by a loss of motility and the method in its present form is as follows: Conidia of *Pseudoperonospora Humuli*, collected from diseased hop shoots, are sown in water. After emergence of the zoospores, samples of the zoospore suspension are transferred to watch-glasses and the material of which the toxicity is to be determined added. The time taken for the complete cessation of movement gives a rough measure of toxicity. It is quite easy to carry out a dozen such tests in an hour, during which time external conditions will not alter greatly. Significant results may be obtained by repetitions co-ordinated by means of a standard fungicide. Results are discarded if the cessation of movement and germination of the control untreated zoospores do not proceed normally. The treated zoospores, in cases where movement does not cease within 5 min. of treatment, are kept and toxic action verified by the ultimate bursting or absence of germination.

If the results obtained by means of this test are to be applied to field conditions, the assumption must be made that infection proceeds through the zoospore stage. It is here that the test differs from that employed by Melhus (17), Uppal (22) and others who treated the conidia and took the concentration necessary to inhibit either zoospore formation or direct germination as their measure of toxicity. Whether the function of the protective fungicide is to prevent germination or zoospore formation or to kill the germ-tube or zoospore is a point which needs investigation.

A test such as the zoospore test described above is really only qualitative, but it serves to illustrate the importance of a factor which occupies rather a curious position in the testing of fungicides, namely, the purity of the chemicals used. The classical example of the influence of impurity is Prévost's (20) observation of the failure of smut spores to germinate in water distilled from a copper alembic. The zoospores are likewise extremely sensitive, and it is so easy for dust or adventitious material to be introduced during the collection of the conidia that the possible presence of impurity must be borne in mind throughout this type of laboratory trial. A trace of protein might vitiate a whole series of trials with copper compounds. One interesting example which has occurred in my work is the effect of a trace of ferrous iron on the fungicidal properties of hydrogen peroxide. Neither ferrous salts nor hydrogen peroxide are highly toxic to zoospores, but if a trace of ferrous iron be added to a non-toxic amount of hydrogen peroxide, the zoospores are rapidly killed. It is possible that a similar action plays a part in the toxicity to various fungi of protocathechuic acid observed by Angell, Walker and Link (1). Solutions of protocathechuic acid and of some other phenolic compounds, if allowed to stand in air under certain conditions of hydrogen-ion concentration, undergo auto-oxidation with the production of hydrogen peroxide. It would have been of great scientific interest if these investigators had excluded from their experiments the possibility of iron contamination, though, under field conditions, it may be assumed that sufficient iron will be present to render protocathechuic acid or hydrogen peroxide highly toxic to copper-sensitive fungi.

*The formation of the toxic agent.* The formation of the toxic agent from the pro-

tective deposit of fungicide is usually ascribed to one or a combination of three reactions brought about, firstly, by atmospheric agencies such as carbon dioxide; secondly, by the intervention of the fungus spore; thirdly, by secretions or other reactions on the part of the host plant.

(1) *Atmospheric agencies.* The study of the decomposition of the spray material and the influence of atmospheric agencies on the chemical changes which may occur after spraying is greatly facilitated by laboratory experiments. Instead of foliage, material such as glass-wool is used to receive the spray, a method which is especially suitable for the examination of the decomposition changes of complex mixtures such as the polysulphide-arsenical-spreader mixtures investigated by Goodwin and myself (8). In spite of the artificial conditions and the many factors left out of account, a number of conclusions which we reached have been confirmed by the general results of field tests. For example, we suggested that the fungicidal properties of the lead arsenate-lime sulphur combination might be greater than those of either constituent, a conclusion in agreement with the results of trials on the hop powdery mildew (9) and with the conclusions, quoted by Hamilton (11), of the orchard tests of Keitt and Wilson against apple scab.

(2) *The intervention of the fungus.* For the study of the part played by the fungus spore in the formation of the toxic agent, *in vitro* experiments are possible. Spores are placed upon the deposit of the protective fungicide and their germination is observed. This method has been so widely used and its technique so improved that many investigators consider it *the* method of determining the toxicity of protective fungicides. The method is undoubtedly of great value as a means of selecting material for field trial, but there are inherent in it many objections which lessen its value. Firstly, there are the difficulties already mentioned in the standardisation of the fungus used; secondly, no account is taken of the possible intervention of the host plant; thirdly, factors which determine the adherence of the spray deposit or dust are ignored. It is, however, because this method has been so widely used and not because of the absence of pitfalls in other laboratory methods that I must choose it for examples of the dangers of neglecting the missing variables in deducing conclusions from laboratory trials. Likewise it is only because it is one of the latest applications of the method that I choose the work of McCallan (15) on the solvent action of spore excretions on protective copper fungicides for these examples. In one series of trials, McCallan placed fungus spores in water in which were immersed collodion sacs containing the precipitate of Bordeaux mixture, the common 0.8 per cent. equal lime mixture. After suitable precautions had been taken, the germination of the spores was examined to see whether spore excretions by diffusion through the collodion had brought about the formation of a toxic agent. But on the plant foliage the spore excretion will act, not upon the precipitate produced by the addition of copper sulphate to milk of lime but upon this precipitate after it has undergone change by drying and by the action of carbon dioxide. Actually McCallan foresaw that the alkalinity of the equal lime Bordeaux mixture might interfere with the reaction and, in some experiments, he used the centrifuged precipitate. But he overlooked the possibility that the chemical composition of the precipitate might change after spraying and it can only have been by accident that the dialysable spore secretion could, in his trials, have acted on a copper-containing deposit similar to that upon which the spore is exposed in the field. Some of the conclusions of

Pickering (19), Barker and Gimingham (3, 7) and later workers who employed the Bordeaux precipitate instead of the Bordeaux precipitate which has undergone weathering are likewise strictly applicable only to conditions immediately following spraying.

In this work, McCallan has made the type of error to which attention was drawn by Tomkins. He judged toxic action by the observation of percentage germination after the incubation period necessary for maximum germination of the control spores. This ordinate is indicated in Fig. 1, and it is evident that only if the germination-time curve is of the type (a) will the relative figures obtained give an approximate indication of toxicity. As McCallan gives no record of subsequent observations of germination, his conclusions concerning toxic action are based upon the degree of retardation of germination after a particular time interval which, of itself, is not necessarily a measure of toxicity.

In offering these criticisms I am aware that they may arise through my incomplete understanding of McCallan's perhaps inadequate description of his work. But these limitations do not apply to the following point. McCallan states in his conclusions that "All the evidence obtained in this investigation appears to demonstrate that the chief agency in bringing about the liberation of copper from insoluble copper protectants is spore excretions." If the meaning of this conclusion is not destroyed by the word "appears," it is that his experiments show that spore excretions play the chief part in producing the toxic agent. Yet all his trials were carried out without reference to the host plant and there is nothing in his tests by which the importance of host-plant secretions can be estimated. How can he conclude therefore that spore excretions are the *chief* agency? Under the conditions of his experiments they were, but to apply in this manner to field conditions, the results of laboratory trials without taking account of the possible effect of the missing variables is to overestimate those results in such a way that their value is seriously reduced.

(3) *The intervention of the host plant.* The examination of the interaction of the protective fungicide and the host plant presents many difficulties and it is not surprising that this problem has received scant attention. Its importance is, however, demonstrated by Barker's (2) observation of the production of hydrogen sulphide from sulphur dusted on the leaves of certain plants. The same investigator has shown that leaves cut from the plant in the usual way are not able to give this reaction. To eliminate the dangerous assumption that the foliage is unaffected by removal from the sprayed plant, I have, in my work on the copper fungicides, gone to the sprayed plant itself. Observations on the production of the toxic agent may be facilitated by collecting from the sprayed foliage the moisture in which the fungus spore must germinate. By the examination of its action on zoospores, the toxicity of this moisture may be determined by a laboratory method which gives results quicker than the final estimation of degree of control and eliminates the variable factor of the incidence of disease. It would appear, since they used the spore to test the toxic agent, that Barker (2), Marsh (13), McCallan and Wilcoxon (16), in their study of the production of hydrogen sulphide from sulphur-dusted foliage, were concerned with the action of sulphur as a protective fungicide. The highly ingenious experiments made and the results obtained by these workers indicate the possibilities of employing the plant itself in the laboratory trial. A possible weakness in the method is that, by enclosing the spore or part of the leaf in an airtight cell, the reactions of the

organism may be modified by this alteration from natural environmental conditions. Although their work indicates the high toxicity of aqueous solutions of hydrogen sulphide to fungus spores in a closed system, it is questionable whether their conclusions may be applied with safety to field conditions, a point which Marsh considered in the interpretation of his results. It is, however, too easy, in the present state of our knowledge, to criticise experimental work and conclusions of the mechanism of toxic action. There are so many biologic factors involved in the investigation of any hypothesis of toxic action that its main virtue lies rather in suggesting new lines of experimentation.

A method whereby the action of atmospheric agencies, host plant and fungus on the fungicide can be investigated under partly controlled conditions has been evolved by the Wisconsin Agricultural Experiment Station (see (11)). The main departure from field conditions of the method is that the variable incidence of disease has been eliminated by the infection of suitable apple trees in pots with an inoculum of ascospores of *Venturia inaequalis* in a special moist chamber under controlled conditions. The treated plants are then placed in a greenhouse and, after an incubation period, the number of lesions per leaf counted. Hamilton co-ordinated the different series of trials by giving the total number of lesions on the unsprayed leaves the relative figure of 100. Although there are insufficient data in his paper to determine the significance of the differences in fungicidal efficiency in each series of trials, and some of his conclusions are based on differences in control which are certainly within the experimental error, repetition under different conditions gave information of sufficient concordance to show the great value of the method for the laboratory examination of fungicidal efficiency in so far as it is independent of the question of the permanence of the protective fungicide on the sprayed plant.

*The adherence of the fungicide.* The determination of the degree of adherence of fungicidal sprays and dusts has been a favourite subject for laboratory investigation. It is generally assumed that with a dust such as sulphur the degree of adherence and even the fungicidal efficiency will be measured by an estimation of degree of fineness. The Chancel test, which determines the volume occupied by sulphur after sedimentation from ether suspension, is an example of this method. As there is general agreement among practical men that the finer the sulphur the better the control, and our work at Wye has proved this in the case of the hop powdery mildew, such methods are of value when applied to sulphur. With copper fungicides, such as Bordeaux mixture, the volume occupied by the precipitate after sedimenting for a definite time has also been used as a measure of adhesiveness or fungicidal efficiency. Pickering (18) stated that "the chief point to be aimed at in making Bordeaux mixture is that the precipitate should be as finely divided as possible and should settle from suspension as slowly as possible." Hawkins (12), Butler (4), de Ong and Root (5), have all employed sedimentation tests to obtain indications of the adherence of the precipitate of Bordeaux mixture prepared in various ways. But the assumption underlying this method entirely ignores the fact that, after spraying, the Bordeaux precipitate will undergo carbonation and changes in its chemical character and physical state. It is therefore not surprising that Pickering found that a Bordeaux precipitate of unsuitable character as judged by the sedimentation test possessed, according to another of his tests, fungicidal properties equal to or better than a Bordeaux mixture prepared with due regard to the voluminousness of the precipitate.



The laboratory investigation of the degree of adherence and permanence of the protective fungicide may be easily accomplished by the analytical examination of sprayed leaves collected from the plant after definite intervals of time. A difficulty is introduced by the growth of the plant and leaves after spraying, and it must be remembered that the procedure gives information only upon the persistence of the fungicide. Deductions of fungicidal efficiency from the results can only be valid if it be shown that the many other factors which govern fungicidal efficiency play an equal or no part.

One further point which may be brought within the scope of this discussion is the frequent failure to give an adequate description of the materials used in both laboratory and field tests. As I have already given examples of this lack of definition in a short communication to *Nature* (14), I need give only one more instance. In a recent number of the *Annals of Applied Biology* (21) the sprays tested for the control of a certain fungus are described by such terms as "0.5 per cent. calcium bisulphite," "0.5 per cent. ammonium polysulphide," "0.5 per cent. colloidal sulphur" and "1.0 per cent. colloidal Bordeaux mixture." In most cases such figures seem to refer not to actual concentration of calcium bisulphite, sulphur or copper sulphate, but to the dilutions employed of certain stock solutions or suspensions. They are, therefore, almost useless without an indication of the composition of the stock material. It is surely obvious that the value of the results of the experiments is greatly reduced by this neglect to give data whereby at least an approximation to the spray applied may be prepared. In spite of the difficulty of giving an unambiguous statement of composition, an adequate analysis can give such information even though, in our ignorance, we do not know the figure or figures which determine fungicidal efficiency.

To conclude this survey of the laboratory investigation of the factors which determine fungicidal efficiency and of the limitations and dangers of the various methods, a brief reply to the question "To what extent can properly conducted and interpreted laboratory tests supplement field trials?" may be attempted. The question rather implies that problems of fungicidal efficiency can be solved by field trials, but I do not think that it is generally realised how great, with the present technique, the differences of control in the field trial have to be in order to be significant. The average field trial is, in most cases, useless for establishing differences in fungicidal efficiency which may be definitely indicated in the laboratory tests and which may be ultimately established by the long run of general experience in spray practice. There is, of course, ample room for improvement in the method of field trial and the variance ascribable to error may be decreased by the elaboration of the trial. But this elaboration of the trial can only be rescued from empiricism by the use of such pointers as are supplied by laboratory tests. From this aspect there can be little doubt of the usefulness of laboratory tests which serve to sift out new fungicides or to analyse problems of fungicidal action. But they are dangerous tools and their value is entirely dependent on the attention paid to the tacit assumptions which they involve.

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## LABORATORY METHODS FOR TESTING WOOD PRESERVATIVES.

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### *Introduction.*

MOST pathologists during the course of their work come up against the problem of testing the relative efficiency of substances which are used for the prevention or cure of the disease with which they are dealing. The animal pathologist is usually concerned with the toxicity to bacteria or protozoa of certain chemicals which may be applied externally or injected into the blood stream. The plant pathologist is concerned only with surface applications of antiseptics which are generally applied not to kill organisms already present but to protect the plant from future attack and to kill any subsequent infection in the form of spores. This protection must be provided for a definite period and the antiseptic must therefore have a certain degree of permanence, which may vary from a few weeks in the case of a spray against a leaf disease to very many years in the case of a wood preservative. Thus, in addition to the fundamental requirement of toxicity or killing power, there is introduced the important factor of permanence. The many other requirements of a preservative, such as the absence of any deleterious effect on the material protected, its harmless nature to animals and man, non-corrosive action to metals, ease of applicability, low cost, and so on, are outside the scope of this discussion but of course must all be taken into consideration when appraising the value of any antiseptic. This paper is limited to a discussion of methods for determining toxicity and permanence.

This problem of the laboratory testing of antiseptics and of how far the results may be relied upon in practice is an acute one in the realm of wood preservation; while 4 or 5 years may be required to give a satisfactory answer from field tests as to the efficiency of a spray, 20 or 30 years may not be enough to prove one creosote more efficient than another, since both will protect the wood for that period.

Some years ago Mr Cartwright and myself were given the problem of setting up a standard laboratory test for the efficiency of wood preservatives, the idea being that it should be possible to get a test which would enable any proprietary or newly invented preservative to be compared to the already well-known preservatives such as creosote or zinc chloride so that it could be said that antiseptic X had a value equal to so many times that of creosote.

Certain methods of test have been laid down, but as yet they are far from being immutably fixed and can only loosely be regarded as standard.

A considerable amount of work has been done to determine the toxicity of various substances to flowering plants but the reaction of these is so widely different from that of fungi that it is of little use to us in the testing of antiseptics.

Clark (2) in about 1900 examined the effect of a number of salts on different fungi and found that small traces of various poisons often had a stimulating effect, a fact well known to be true of other organisms and which has often been confirmed since. Malenkovic was one of the first to test wood preservatives in the laboratory, but his methods were somewhat crude; he exposed beakers of gelatine medium containing the antiseptic to the air for 14 days and noted whether they became mouldy, no attempt being made to use pure cultures. Since that time successive investigators have made tests of antiseptics and wood preservatives upon the most varied media, such as various natural and synthetic agar and gelatine media, toasted bread, rice, sawdust and wood. Some of these tests have been very carefully carried out with the use of pure cultures of fungi, but unfortunately the results obtained by different workers, though in themselves quite reliable, are in no way comparable and of very limited application.

Humphrey (5), Richards (7) and other workers at the United States Forest Products Laboratory at Madison have made a very large number of tests, using the Petri-dish method, in which small pieces of fungus are cut from a growing culture and placed upon a series of plates containing medium with increasing concentrations of antiseptic. By carefully standardising their conditions they have been able to obtain consistent results when only one species of fungus has been used for making the tests.

Owing to the variety of methods in use in different laboratories in the United States, a conference of the workers in this field was held at the Missouri Botanic Gardens in December 1929, which came to an agreement and laid down what was considered as a standard test, specifying the medium to be used, the test fungus and the details of procedure. These were published by Schmitz (10) in 1930.

Shortly after this meeting Dr von Schrenk came over to Europe and invited a number of European workers to meet in Berlin and discuss laboratory methods of testing wood preservatives. At this conference it was immediately evident that the European workers, who were mostly from Germany, held widely different views to the American, in that they regarded the agar test as one of doubtful value and were unanimous in recommending the use of wood as a medium (1). A number of very interesting contributions were made to the meeting and they served in particular to

emphasise the extraordinary variety of methods that are in use and the widely divergent results that may be obtained. Criticism was particularly directed at the use of a single fungus for making tests.

*Agar tests.*

A description of the technique employed at the Forest Products Research Laboratory, Princes Risborough, for carrying out an agar test is given below.

The medium, which contains 2 per cent. Kepler's malt extract and 2 per cent. agar, dissolved in distilled water, is sterilised in an autoclave at 15 lb. pressure and measured quantities of this are poured into sterilised bottles or flasks containing varying amounts (by weight) of antiseptic in a fixed volume of water. It is absolutely essential that the medium should *not* be sterilised in contact with the preservative, as all kinds of reactions may take place and the toxic point obtained may be very different from that obtained when they are sterilised separately. Humphrey and Fleming (5) recommended that the medium and the antiseptic after measurement should be sterilised separately in glass stoppered bottles held in a clamp and then mixed, but this method has been found responsible for heavy casualties in glassware. By careful working, contamination of the medium, when measuring it out, can be avoided, and a second sterilisation is therefore unnecessary. The antiseptic solution usually need not be sterilised, but if an extremely dilute solution, of a strength insufficient to kill mould spores is required, this may be prepared by adding a small quantity of a strong solution to sterile water. Very frequently the substance which it is wished to test is of an oily nature and not miscible with water, in which case an emulsion of it must be prepared.

Various emulsifiers including soap, turkey red oil and gum arabic, have been used; the last is the one which has proved most satisfactory, since it is in itself completely non-toxic. Sometimes, in order to get satisfactory emulsions of substances such as tars and creosotes, a high speed stirrer must be used.

Care must be taken in selecting an emulsifying agent to avoid the use of a substance which is likely seriously to alter the toxic point of the substance under test. Rabanus (6) has shown how the addition of small amounts of oil to a culture medium greatly reduces the toxicity of chlorthymol to moulds.

The medium and the preservative are well mixed, poured into a Petri dish and, when solidified, inoculated with a small square transplant from a plate culture of a wood destroying fungus. The plates are examined for growth at weekly intervals up to 4 weeks and the toxic point is given as the interval between that concentration on which growth just takes place and the concentration next above it at which no growth takes place. Growth is defined as growth from the transplant which is visible under a hand lens.

In all toxicity work it is very important to continue observations over a fairly long period and to fix a sufficiently long standard time for the tests, because the action of many preservatives is to retard growth and not entirely to prevent it. Dr Tomkins, at a recent meeting of the Mycological Society, brought forward a series of curves which illustrated the retarding action on the germination of mould spores of various lethal agents and emphasised the importance of continuing observations upon toxic effect over a definite period. His work refers to spores, but exactly the same effect has been found with transplants of mycelium; quite frequently there is

not the slightest sign of growth till 3 or 4 weeks after inoculation of the plates. Whether or not the transplant of mycelium has been actually killed may be readily determined by transferring it to a tube of fresh medium and incubating it for a few days.

There are a number of sources of error inherent in the agar test, the medium has to be measured at a temperature over 50° C. and most laboratory glassware is graduated for use at 15° C. Then again the medium is viscous and a certain amount remains on the walls of the burette; 0.5 c.c. is allowed for this, but that is only a rough correction because the actual amount left on the glass depends on the temperature of the liquid and the speed at which it is run out. Many and varied refinements of the method have been suggested, but I do not think it is worth seeking to refine this method too far since the results which it gives may not tally with the results obtained on wood and I regard the agar method rather as a preliminary test to eliminate the useless substances. The reasons for this view will be given later after describing the wood block tests.

#### *Wood block tests.*

In principle the wood block test is simple—a piece of wood treated with a definite concentration of preservative is exposed to vigorous fungus attack under controlled conditions and the result noted.

The technique of the wood block test has been developed by Falck and the workers at the Laboratory of the Rutgerswerke Company in Berlin and a description of it was published by Falck in 1927 (4).

Some of the snags and difficulties that arise in carrying out a wood block test may now be considered. First and foremost there is the question of obtaining a uniform medium, wood is a notoriously variable substance, it is perfectly true to say that no two pieces of wood are ever exactly the same. The variations in the chemical and physical nature of specimens of wood from trees of the same species is amazing. One specimen may be dense, slow grown and extremely resinous and in consequence resistant to fungus attack, while another may be light, quickly grown, almost free of resin and readily decayed by fungi. Great care must therefore be taken to select wood that is not only of the same species but is as far as possible typical of the species and of average rate of growth and so on. In making determinations which shall be internationally comparable we are at once up against the difficulty that the same tree species do not occur in all countries. In Europe it may be agreed to use the sapwood of Scots pine (*Pinus sylvestris*) and of beech (*Fagus sylvatica*), but in the United States of America Scots pine does not occur and it is said to be almost impossible to distinguish with certainty between the woods of the different species of pines resembling it—the Southern pine and the Pitch pine groups.

The next problem is to get the various concentrations of preservative uniformly distributed throughout the wood; in the case of water soluble salts this may be achieved by fully injecting the wood under vacuum and pressure with a solution of the preservative and allowing it to dry out slowly, a certain amount of mass movement does take place increasing the concentration at the end grain, but this does not seem important.

In the case of oily preservatives not soluble in water, the class to which creosote, the most important wood preservative, belongs, the problem is much more difficult.

There are two ways in which the oil may be introduced into the wood in small quantities: (1) in a neutral solvent, (2) in emulsion with water.

When using a neutral solvent one is always faced with the difficulty of completely removing this before exposing the blocks to fungus, since any traces of a solvent such as petroleum ether will in themselves have quite an appreciable toxic action.

So at Princes Risborough, the second method is the one principally used, the blocks being injected under pressure with dilute emulsions kept vigorously stirred with high speed centrifugal pumps. Undoubtedly the emulsion does tend to break up on entering the wood, but in dealing with a small piece the penetration seems fairly even. A thin layer of carbon may be deposited on the outside of the block, but as this is non-poisonous it is without significance.

Some workers have used sawdust as a medium in order to get a more even distribution of the preservative, but this makes the control of the moisture content difficult since fungi require two or three times as much water in sawdust as they do in wood. It is also impossible to infect the sawdust in the way that a block can be infected, by placing it on a growing culture, and lastly it is difficult to observe the amount of attack upon sawdust. Curtin (3) mixed powdered wood with an agar medium, but this seems merely to complicate the agar test without introducing any advantages.

It is somewhat easier to get the blocks to a suitable moisture content for growth after treatment with emulsions than after treatment with the preservative in a neutral solvent. The treated blocks are suspended in a cage on a spring balance in an oven and allowed to dry at 50–60° C. till they reach fibre saturation point, i.e. till all the free water from the cells has evaporated and the moisture content is about 30 per cent. of the dry weight. They are then inoculated by placing them upon actively growing cultures in a special culture flask. The flasks are kept for four months in an incubator at a temperature a little below the optimum for the growth of the fungus. At the end of this period the blocks are removed from the flasks, examined for fungus growth, oven dried and re-weighed. The difference between the final weight and the initial weight gives a useful measure of the amount of attack. An example of a definite end point obtained in this way may be given.

Blocks of beech impregnated with emulsions of a creosote distilled from low temperature tar were exposed to *Polystictus versicolor* for four months and the following losses in weight occurred:

0.5 per cent. creosote—loss in weight 38.4 per cent.				
1.0	„	„	„	37.1 „
2.0	„	„	„	0 „

In this case we can say that the toxic point lies pretty definitely between 1 and 2 per cent. Another difficulty with which one has to contend is the contamination of the blocks with moulds, since it is difficult to avoid contaminating the blocks during the various treatments which they have to undergo after the original oven drying sterilisation. A little contamination may not greatly affect the growth of wood destroying fungi, but as soon as mould becomes excessive it tends to check the growth of the Basidiomycetes.

*Species of fungi to be used for tests.*

In considering the species of fungi to be used in carrying out tests of toxicity the position is rather different from that of the plant pathologist who is dealing with a specific disease caused by a certain organism. He will of course have to carry out his tests using that organism. Extremely erroneous results may be obtained by using a different class of organism from that against which it is desired to protect the plant. In the case of timber decay it is necessary to protect against a whole range of fungi which vary greatly in their action on the wood and in their reaction to antiseptics. This point has not been sufficiently realised by some workers, who are quite content to base all their conclusions upon results obtained with a single strain of one species of fungus. In America the desire for simplification and standardisation of the methods of test has led to the use of a single species, *Fomes annosus*, which is claimed to be specially resistant to antiseptics.

Richards (8, 9) tested the resistance of a large number of species to sodium fluoride and zinc chloride and found *Fomes annosus* to be about the most resistant species, but she observed considerable differences in the reaction of the different fungi to the two antiseptics.

The desiderata for a test fungus should include the following:

- (1) It shall be a rapid and active destroyer of felled wood.
- (2) Resistant to fungicides.
- (3) Of economic importance.
- (4) Not unduly sensitive to slight variations in environmental conditions.

*Fomes annosus* is resistant to antiseptics but is equalled in this respect by other fungi. It cannot be regarded as a particularly active wood destroyer since it does not cause rapid loss in weight and for this reason it is unsuitable for use in wood block tests. Except in coal mines it is not of economic importance in the decay of timber, occurring more frequently as a parasite of living trees. It is responsible for a so-called white rot, whilst all the most important decays of coniferous timber are of the brown rot type.

The fungi which we use in our tests include *Polystictus versicolor* on beech; *Lentinus lepideus* and *Coniophora cerebella* on Scots pine, and these fungi together with *Fomes annosus* for the agar tests.

It is essential to use several fungi for all tests, since no one fungus is consistently the most resistant to all preservatives. In a recent paper by Rabanus (6), the extraordinary variation shown by different fungi in their reactions to preservatives is illustrated graphically in a figure which is reproduced here.

The toxic points of a number of different substances against various wood-destroying fungi are plotted on a logarithmic scale. All the fungi tested were wood-destroying Hymenomycetes, and had fungi from other groups, such as moulds, been included, the variations would have been much wider. Species of *Penicillium* and its allies will grow vigorously over fully creosoted timber which is absolutely immune to decay causing fungi, and there is a pinkish mould which *prefers* to grow on wood painted with Solignum. There is no need to elaborate this point; the amazing vitality of our fungus weeds is only too well known and it is generally recognised that toxicity tests of wood preservatives must be carried out with wood-destroying fungi. What is perhaps not so well recognised is that the variation *inter se* of wood-destroying fungi is such that conclusive results cannot be obtained by the use of a single fungus. Nor

are the fungi consistent in their resistance to antiseptics: *Coniophora* which is sensitive to arsenic is resistant to copper but for *Schizophyllum* the position is reversed.

Some compounds, such as sodium fluoride, are about equally toxic to all the fungi tested, towards others such as mercuric chloride the organisms show widely varying resistance. This variation in resistance to poisonous substances is also shown in a lesser degree by different strains of the same fungus and an endeavour is now being

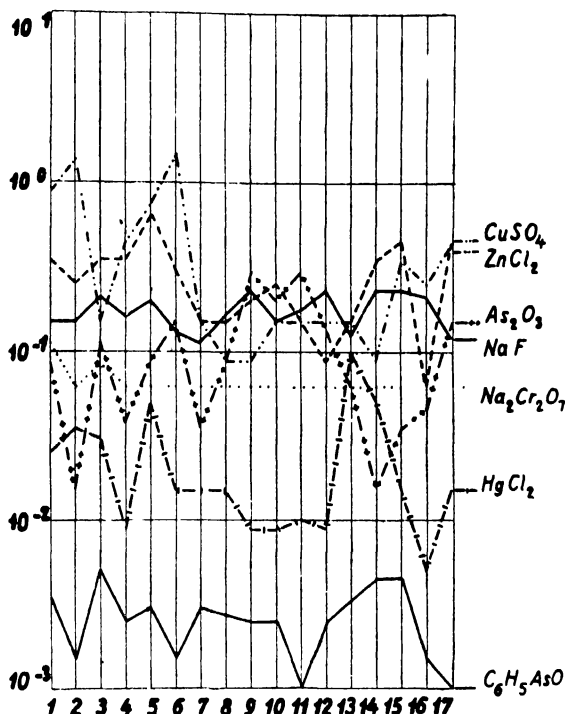


Fig. 1. Merulius lacrymans. 2. Coniophora cerebella. 3. Polystictus versicolor. 4. Ptychogaster. 5. Polyporus vaporarius. 6. Polyporus sulphureus. 7. Polyporus Schweinitzii. 8. Lenzites abietina. 9. Lenzites thermophila. 10. Lenzites trabea. 11. Lenzites saepiaria. 12. Pleurotus ostreatus. 13. Stereum purpureum. 14. Schizophyllum. 15. Fomes annosus. 16. Trametes pini. 17. Lentinus lepideus.

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made to come to some agreement among the different workers as to the strains of different test fungi to be used, and it is hoped that eventually all the laboratories concerned will use fungi from the same isolations.

#### *Relation between values obtained in agar and in wood.*

Now let us compare the results obtained from agar and wood block tests. In a few preservatives these tally quite closely; the percentage of sodium fluoride required to prevent growth of wood destroying fungi upon wood and in malt agar is roughly the same, but in the case of mercuric chloride the concentration required to inhibit



growth in wood is on the average ten times greater than that required in agar. Some recent tests which we have carried out upon the toxicity of various creosotes have shown that a similar sort of relation occurs, the percentage required to prevent growth upon wood being in the neighbourhood of ten times the amount required in agar. But the relation varies for each antiseptic and to a certain extent for each fungus, so that we cannot deduce any factor from which we can calculate the toxic point in wood from the toxic point in agar. The critics of the wood block method, mostly from America, point out all the sources of error in the wood block test and admittedly these *are* many and in a bad example these may perhaps amount to 25 per cent., but is that to be compared with an error of 1000 per cent. or more which is what we are really getting when deductions are drawn from an agar test of mercuric chloride? The wood block test has errors which careful working may reduce, the agar test has errors which are fundamentally inherent in it and which no amount of care can possibly reduce. For that reason super refinement of the agar method can only be regarded as a mere waste of time. But I do think that the agar method has its uses, especially if it be carried out with a number of fungi, in providing a rough idea of toxicity and it can be used fairly safely to compare substances of the same general chemical nature. For instance, the relative toxicity of a series of tar acids differing only in their boiling points may be fairly accurately gauged from agar tests but no comparison of the value of mercuric chloride, arsenious acid, and creosote can safely be made from agar tests.

Now let us consider reasons for these discrepancies. Take first the physical condition of a salt in an agar medium, it probably almost completely dissociated but the presence of electrolytes and of much colloidal matter may act as a buffering agent, while diffusion is rapid and easy. In wood, on the other hand, ionisation and diffusion are probably both considerably less. From the chemical point of view the reactions between the preservative and the mixture of colloidal organic materials in malt agar medium cannot be the same as the reactions that take place with wood, which is an entirely different mixture of colloidal organic substances. In a few instances there is some evidence of the reactions that are taking place. Mercuric chloride is known to become fixed in the fibres of the wood so that a proportion of it becomes less soluble in water. Some of it is evidently withdrawn from the sphere of action by adsorption on and absorption by the cellulose and other constituents of the cell wall. Hence the toxic point in wood is considerably higher than that in agar, a certain amount of the preservative having been thrown out of action in the wood. There is no need to labour this point; it is obvious that the reactions between the antiseptic and an agar medium and between it and wood must be vastly different. Then again there are substances possessing little or no toxicity which may so alter the physical conditions in a piece of wood that no fungus is capable of growing in it. A substance such as a wax or varnish which shows no preservative action in an agar medium yet might so alter the wood physically as to render it impervious to water and immune to attack by fungi.

The different methods of inoculation in the agar test and the wood block tests may also explain in part the different results obtained; in the former a small amount of mycelium is transferred to a poisoned medium, while in the latter, a treated block is exposed to the attack of a relatively large amount of fungus mycelium. Rabanus (6) carried out agar tests in which small pieces of medium containing the preservative

were placed upon fungus cultures in flasks; in this case the proportion of fungus mycelium to preservative was greater than when the test is carried out in the normal way and it was found that considerably more preservative was required to prevent growth upon the poisoned medium than when the test was carried out in Petri dishes.

There has been little work from the chemical side on the reactions between different woods and preservatives, though much data as to the relative permeability of timbers to antiseptics has been collected. There is no evidence to show that an antiseptic may be much more toxic in one wood than in another, but this possibility must not be overlooked. The figures obtained by Rabanus and at the Forest Products Research Laboratory for the toxic points in beech and in Scots pine do not differ very widely so that reliable results may be expected if two readily decomposable timbers are used, one a dicotyledon and the other a conifer. The important thing is to use a timber of low natural durability and one on which the test fungus makes good growth.

#### *Value of laboratory tests.*

Now comes the important question of how far the results of laboratory tests are applicable to field conditions. It is not possible to give a precise answer to that question yet, as unfortunately no one was carrying out wood block tests upon the comparative toxicity of the various creosotes which were used to treat the railway sleepers and telegraph poles of which we now have durability records running into 30, 40 or 50 years. However, the results of laboratory tests can be compared with what has been generally found in practice and with what has been found in other countries, particularly in the tropics where the processes of decomposition are more rapid than they are in this country.

As was stated at the beginning of the paper, the two main factors contributing to the efficiency of a preservative are (1) toxicity, (2) permanence.

If the substance is sufficiently toxic to kill any fungus which comes into contact with the wood, it is certain to protect the wood from decay and it will continue to do so just so long as the concentration in the wood is sufficiently high to prevent fungal growth.

Nothing has been said as yet about standard tests for permanence, *i.e.* for resistance to leaching and weathering, a problem that is under investigation at the moment. Long continued simple washing with changes of water is an efficient method of removing the soluble material, but is not exactly comparable to field conditions where the treated wood is exposed to that rapid alteration of wet and dry, cold and heat that we know as "weather," and where also interactions with the soil may play a part. One French worker has made resistance to leaching his main criterion for judging the value of a preservative and he compares antiseptics on the basis of the number of times which it is necessary to wash the treated wood before it becomes susceptible to decay.

Though there is something more convincing, especially to practical men, about the results from field tests than from laboratory experiments, yet they are open to quite as many sources of error. In our so-called "graveyard" tests, in which treated blocks are half buried in the earth, soil heterogeneity may cause irregularity in the results: one batch of samples may chance to be placed upon the site of an old manure heap and so be exposed to increased infection. By having three such "graveyards,"

one at Princes Risborough in the heavy soil there, one at Thetford on a sandy soil and one on the side of a Welsh Mountain on peat, this source of error will be minimised. In the true service trials that type of source of error is even more prominent—take railway trials of sleepers, a half mile may be laid with sleepers treated in one way and the next half mile laid with sleepers treated in another way, the soil conditions may not be the same in those two half miles.

Field and service trials are outside the province of this discussion but it must be emphasised that they may not give such reliable results as one might expect, and in any case the results are applicable only to that set of conditions under which they were tested. The ideal test for any preservative is exposure of the treated material to deterioration in the situation in which it will be used in practice. Substances which it is proposed to use for the preservation of mine timber should finally, of course, be tried upon pit-props in a damp mine, but results from laboratory tests would be quite, if not more valuable in adjudging the value of these substances than results from field trials in the open.

The intensity of fungal attack upon a treated piece of wood in one of our flasks is much greater than in the open and, neglecting for the moment the question of leaching, the values obtained in the wood block test should prove effective in practice. Rabanus (6) considers that the values obtained in wood block tests are scarcely ever too low to apply directly to practice and quotes the case of copper sulphate, of which in one case over 6 per cent. was required to prevent fungal growth in a wood block test, while poles treated with a lower concentration stood perfectly undamaged in the open for 10 years. I am inclined to disagree with him here and suggest that the concentration to be used in practice should be from 5 to 10 times (depending upon the amount of leaching that will take place) the toxic limit found in the wood block test.

Summarising what appear to be some of the important points to be considered in making laboratory tests upon antiseptics to be used in the protection of materials:

(1) The experiments must be carried out with pure cultures of the organisms against which it is wished to protect the material. In the case of wood preservatives, a number of fungi must be used.

(2) The medium used must be the material which it is desired to protect, in as nearly as possible the normal physical condition.

(3) Conditions for the growth of the injurious organism must be optimum.

In conclusion one may express the opinion that when once we have arrived at satisfactory tests for toxicity and leaching, it should be quite possible to predict accurately the effectiveness of wood preservatives from laboratory tests.

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## PROCEEDINGS OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS. II.

ANNUAL GENERAL MEETING held on Friday, February 26th, 1932, at 2.30 p.m., in the Botanical Lecture Theatre of the Imperial College of Science and Technology, London. The Chair was taken by the President, Dr A. D. IMMS, F.R.S.

After the business a discussion was held on "Laboratory Tests of Insecticides" in which the following papers were read:

- I. Laboratory Methods for Evaluating Insecticides. By F. TATTERSFIELD, D.Sc., F.I.C., of the Rothamsted Experimental Station, Harpenden, Herts.
- II. Laboratory Tests of Insecticides for use against Wood-Boring Insects. By F. R. CANN, D.I.C., of the Forests Products Research Laboratory, Princes Risborough, Bucks.

### I. LABORATORY METHODS FOR EVALUATING INSECTICIDES.

By F. TATTERSFIELD, D.Sc., F.I.C.

*(Rothamsted Experimental Station, Harpenden, Herts.)*

IN a recent discussion before this Society of the means of testing fungicides in the laboratory, it was pointed out that such tests are only preliminary antecedents of field tests. The same consideration applies to the laboratory tests of insecticides. It is not, I think, suggested that they will give full and complete information of the economic value of any particular chemical compound or plant product; before that can be decided many factors have to be taken into account, such as ease of application, safety in use both to the plant and to man, cost of production, etc. Laboratory trials, however, form an almost invaluable adjunct to field trials, and may effect an enormous saving in labour, time and money in selecting the more potent materials for trial on a larger scale.

At the previous discussion the difficulties inherent in methods for testing fungicides were treated with some completeness—they were shown to be many and varied. The difficulties of testing insecticides are no less real but perhaps less varied, and workers on the subject are by no means satisfied that the last word has been spoken on the subject, with the result that year by year new suggestions are made and new apparatus and methods evolved.

There are one or two differences between the means of testing fungicides and testing insecticides. The test subjects in the former are microscopic and may be obtained in large numbers and worked on in quantities suitable for statistical treatment; in the case of the latter the test subjects, except in the case of insect eggs,

are more or less macroscopic and fewer numbers have often to be dealt with in laboratory trials; moreover, in order to obtain adequate supplies fairly large apparatus is required. It is not my province to deal with this very important side of the subject. I may perhaps remark that it is fortunate that a large range of insect pests can be separated for a period of time from their hosts without material injury and a greater degree of freedom is possible with them than with fungi. Insects, however, are on the whole more complicated structures than fungi, and it is often difficult to evaluate both quantitatively and qualitatively the effect of the administration of the insecticide. Both qualitative and quantitative aspects of the question are important. Reference to this will be made later. The various forms of apparatus and the diagrams I shall show are elaborated to give some quantitative expression of the results of insecticidal action.

I propose to deal with the subject firstly, by showing slides of forms of apparatus with a brief description of the methods employed, secondly, by showing slides of diagrams that summarise the results so far obtained by certain workers in this field and, thirdly, to attempt very briefly and perhaps inadequately to discuss some of the difficulties involved in this class of work.

Insecticides are usually divided into two classes, according to their mode of administration: (1) stomach poisons, a self-explanatory term, (2) contact poisons—a thoroughly bad term, but one which simply implies that the insecticide is applied externally but acts lethally by penetration to some vital part.

(1) *Stomach poisons.* Despite a considerable amount of work, no simple and entirely satisfactory method has so far been evolved for testing stomach poisons. The older workers, including Lloyd, Gimingham and myself, employed what one can call the cage method. Lloyd (17), in his valuable investigation of the tomato moth (*Hadena oleracea*) and its control, sprayed small bushy plants in pots with arsenate of lead of varying concentrations; when dry the plants were infested with a large number of more than half-grown larvae, the plants being afterwards enclosed in muslin sleeves; each day the numbers of dead larvae were counted. In this way he was able to determine approximately the rates at which the insects were being poisoned by the different concentrations. I have graphed some of his results which I propose to show later.

The method evolved by Gimingham (16) for testing sodium fluosilicate and certain plants as stomach poisons involved spraying shoots with foliage with certain concentrations of the insecticides. The foliage after being allowed to dry was enclosed in hurricane-lamp glasses, the stalk dipping into a test-tube of water. A number of larvae were put on the foliage and kept from escaping by muslin stretched on an iron ring. The insects were observed from time to time and the number of dead determined. The two methods are very much alike and they give very valuable information, if a careful inspection of the foliage is made from time to time and a record kept of the feeding of the insects. Thus it was shown that the fluosilicates are potentially valuable insecticides and that certain of the fish-poison plants act as powerful repellents to certain insects. The two methods may be regarded as semi-quantitative only, as there is no means available of ascertaining the dosage actually taken by the insects.

Diagram 1 shows the data of Lloyd graphed in order to show percentage deaths with time. The results are remarkable, as there is no presumptive assurance of the

doses ingested by the insect being proportional to those sprayed on the foliage. The curves are typical for this kind of work and show the tailing-off effect as the 100 per cent. death-point is approached. Despite all criticism that could be levelled on theoretical grounds, these data are of great value, for they demonstrate that under conditions closely approximating to those of large-scale practice there is little to be gained by applying doses of poison beyond a certain concentration. Coupled with observations of the amount of foliage eaten, Lloyd's figures constitute most valuable information on the toxicity of lead arsenate to the larvae of tomato moth. It is, however, questionable if, applied over a range of compounds, this method would be suitable for purposes of obtaining numerical comparisons of toxicity. The cage method is nevertheless one that might well be interposed between more refined but highly artificial laboratory methods for testing stomach poisons.

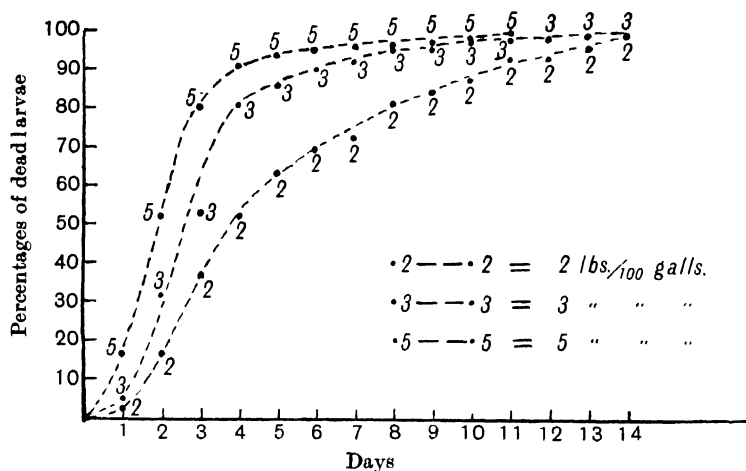


Diagram. 1. Death-rate of larvae of tomato moth on plants sprayed with lead arsenate (from Lloyd's data).

The chief worker in this field, F. L. Campbell, has attempted to introduce more refined methods with considerable success. Their drawback, if any, is their laborious nature, which he readily admits. In his first method he fed larvae by hand from droplets containing known quantities of poison, and determined the speed of toxic action. In this way he was able to express graphically the relative toxicities of tri- and pentavalent arsenic (1, 2) and later to compare the susceptibilities of different larvae and of different instars of the same larva. He has more recently elaborated an apparatus for testing dusts by means of what he terms the sandwich method. (*Here a slide of Campbell's apparatus was demonstrated.*)

Campbell and Filmer (3) blow dusts into an inverted jar and after the larger particles have settled, place the jar over a glass plate upon which circular cover-slips and circular discs of mulberry foliage of the same size are distributed; the fine dusts settle uniformly over the plate. After a suitable interval of time the plate is removed, the amount of dusts deposited on each disc is determined by weighing the cover-

slips, and then leaf sandwiches are prepared by placing on each poisoned leaf disc a disc of foliage, wetted with starch paste, the two being finally pressed together. The discs are inserted into split corks and silkworms allowed to feed on them. Individual dosages are varied at will by permitting the larvae to eat varying amounts, the amount of leaf eaten being finally determined by means of a planimeter. Campbell (3, 4) attempts to determine (1) what he terms the "knock-out" point, i.e. the time taken to paralyse the insects' powers of regaining their feet when pushed over; (2) the time required to kill. He finally divides his results into three categories (a) sublethal, (b) intermediate and (c) lethal, and plots the reciprocal of the time required to achieve either the moribund state or death, i.e. the speed of toxic action against the dosage in milligrammes per gramme body weight. (*Here were shown two diagrams giving Campbell's results.*)

✓ Campbell seems to agree that what Trevan (5) has termed the "Median lethal dose," a phrase equivalent to our 50 per cent. death-point or concentration required to kill 50 per cent. of the test subjects, is the most suitable for estimating toxicity, but that it does not tell all the story of toxicity. He measures toxicity by determining the areas under his curves by means of a planimeter. The method is arbitrary, but is excused by the amount of labour required to ascertain the median toxic dose. Campbell prefers the moribund or "knock-out" curve as giving more precise results. The question arises: is the greater exactness of the data due to the fact that in this condition his insects are half dead or thereabouts, and he is thus determining the rate to half-kill approximately his insects? I use the word half-kill very loosely. Campbell, by taking this stage as his end-point, is avoiding in all probability the great variation in dosage that is likely to occur as the death-point is approached. The method seems capable of giving results of value, but it is slow and rather tedious and it would be necessary to eliminate by preliminary trial all but the most toxic or economically most feasible compounds. There is an obvious need for a simplification of method but it is difficult to see how it is to be introduced for the examination of stomach poisons. Campbell is adding a good deal to our knowledge by his attempts to introduce greater precision into our methods, and his plea that at present it is unnecessary to determine "median" lethal doses with a high degree of exactitude is probably justified. At a later stage for a few of the more important compounds it might be justifiable to spend time and effort in attempting to ascertain with as great an accuracy as possible these constants (if they are "constants") of toxicity.

*Contact insecticides.* The difficulties of evaluating contact insecticides are not so great as those associated with stomach poisons, at least superficially, as it is easier to apply poisons externally than to administer them internally. The two general principles usually applied are (1) to spray a definite volume of fluid containing known concentrations of poison on to a surface upon which the insects are placed with the assumption that the amount falling on each insect will be proportional to its surface and probably to its body weight; (2) to spray known volumes into containers in which the insects are flying about, with the same assumptions. The insects sprayed being generally of nearly the same size will receive in both cases approximately the same doses for the same concentrations. As it is customary to compare toxicities of different compounds with reference to a particular insect, the assumptions are not likely to be far from the truth. (*Here were shown slides giving three different instruments*

used<sup>1</sup>). The object in the main is to spray under as constant a set of conditions as possible, but it is well to point out here that little is known precisely of the effect of pressure, temperature and of globule size on toxicity, or how far the resistance of the insect depends upon meteorological conditions prevailing before and after spraying. The insects after spraying are observed and toxicity is evaluated either by determining the time taken in rendering the insect moribund or dead for each concentration or by the numbers rendered moribund or dead for each concentration of poison after a time sufficiently long for the full effect of the poison to come into play.

H. H. Richardson (8) has recently published an account of an improved apparatus for spraying house-flies with kerosene-pyrethrum solutions. Similar methods have been described and used (9), but Richardson has secured considerable uniformity with respect to temperature, pressure and other conditions, and he lays down precise recommendations for use. Fifty to sixty house-flies of the same age (reared under constant conditions) are introduced into a wire screen cage through an opening at the side. The box is tightly closed and the fan switched on and 1.6 c.c. of spray is atomised into the cage where it is distributed by the fan. At 30 sec. intervals, counts are made of the number of paralysed flies lying on the clean brown paper on the bottom of the box. The counts are continued until well over 50 per cent. of the flies are down. Afterwards the flies are counted and transferred to a previously prepared cylindrical wire cage. After 24 hours counts are made of the dead and live flies.

Before passing on to a discussion of data, I might mention an alternative method of evaluating contact insecticides, namely by dipping. This method has been used by us in an examination of the toxicity of *Derris* root (10) and has recently been re-investigated by H. H. Shephard and C. H. Richardson (11). Insects are dipped for a known length of time in solutions or suspensions of the poisons and the mortalities compared with the concentrations. These authors consider that, in view of the fact that spreading and wetting reagents are unnecessary in the dipping method, it has an advantage over spraying methods. Personally, I cannot see the force of this criticism, for in both cases it would appear necessary to bring the insecticide into as close a contact with the insect as possible and, provided a non-toxic wetter is used and always used at the same concentration, it would appear to me to be an advantage in both methods and to approximate more nearly to large-scale usage from which one should not be too remotely removed. Our experience has been that dipping methods are difficult to carry out without damage to the insect, and that the method is prejudiced seriously by complete absence of knowledge as to how far effects other than the toxicity of the compound are playing a part.

I have described these methods together, as I wish to present together the kind of data obtainable. Before proceeding to do so I might quote with advantage a note appended by Dr R. A. Fisher to a paper by H. M. Morris and myself (6):

"In any given experimental conditions the probability of any particular insect dying must be regarded as a continuous function of the concentration of the insecticide used. The control gives any experimental value of the probability of death corresponding to zero concentration, and with any effective insecticide we must imagine that as the concentration is increased, the probability of death increases

<sup>1</sup> The apparatus of Tattersfield and Morris (6); the apparatus of O'Kane, Westgate, Glover and Lowry (7); the apparatus of H. H. Richardson (8).



from this minimum value, until possibly a final concentration is reached at which death is certain.

"The relation between concentration and probability of death could theoretically be determined by experiment by exposing a large number of insects to the action of the insecticide at each concentration. The number of insects required, however, increases enormously if we wish to explore in this manner the region in which the probability of death is high. If as many as 99 per cent. of the insects were killed, the accuracy of the comparison between any two insecticides would depend upon the comparatively few insects which survived, and to compare them with any accuracy many thousands of insects would have to be used. The same difficulty arises in the comparatively unimportant case when the deaths are few. For a given number of insects the most accurate comparisons can be made when the concentrations are such that about 50 per cent. perish. The region between 25 per cent. and 75 per cent. can be fairly easily explored. It is for this reason that the preliminary examination of chemical substances should be made by a comparison of the concentrations required to give a mortality of 50 per cent.; when the equivalence at this point is

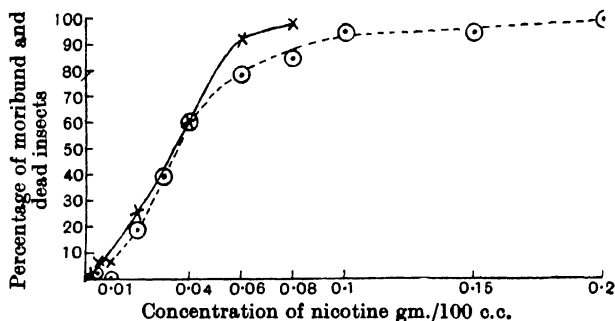


Diagram 2. Characteristic curves for toxicity of nicotine to *Aphis rumicis*.

established, it would further be most valuable to ascertain if the same relative concentrations are equivalent over the range 25–75 per cent. Only in this way does it seem possible to infer a general equivalence of insecticidal properties. The direct comparison of mortality when the probability of survival is very small would seem to be beyond the scope of accurate laboratory investigation."

Diagram 2 summarises the results obtained by Tattersfield and Morris in investigating the toxicity of nicotine to *Aphis rumicis*, fifty adult wingless females being sprayed ten at a time to determine each point; two series of tests were made on separate days. The curves are S-shaped. The mean of the two sets of results might be described in Trevan's phrase as the characteristic of the toxicity of nicotine to *Aphis rumicis* and their most notable feature is the tailing-off shown as the 100 per cent. death-point is reached. The 100 per cent. death-point is approached asymptotically and, except in very fortunate circumstances, would appear very difficult to determine with accuracy. I have later to indicate what I believe was a series of experiments where such a fortunate circumstance seems to have occurred. The most noteworthy feature of these curves is their close approach round about the "median" toxic dose, i.e. the concentration giving 50 per cent. of deaths. The same tailing-off

effect is shown in some data accumulated by Gimingham, Massee and Tattersfield (18) in studying the toxicity of dinitro-cresol and its sodium salt and a tar-distillate spray to the eggs of *Selenia tetralunaria*.

The S-shaped curve is common in this type of work. Its significance for these investigations has been brought out in two important papers by Henderson-Smith (12) which should be read in full by all workers in this field. He has shown that curves of this nature are most readily explained by variations in resistance of the organism to the action of the killing agent and the type of resistance distribution curve. Shackell (14, 15) also, in his studies of the relationship between dosage and effect, found that it was represented by an S-shaped curve and that this curve sums up or integrates the frequency distribution in sensitivity (or resistance) among the organisms used. He assumes, further, that if it were not for inevitable differences in sensitivity (or resistance) among the organisms used, the relationship between dosage and effect could be accurately described by a straight line. Again he concludes that as a consequence no mathematical function, the graph of which exhibits a steep slope in the region of the minimal dosage (e.g. a monomolecular curve) can be used to describe this relationship. Henderson-Smith (12a) has shown that a so-called monomolecular survivor curve is only obtained when the frequency curve is slightly skew.

In H. H. Richardson's paper (8) we notice a similar set of results when the percentage number of flies paralysed is plotted against the time, and he considers that the principle is established by his figures that the most accurate comparisons can be made between various strengths of insecticides when the concentrations are such that 50 per cent. of the insects are paralysed.

I am not competent to deal with the statistical side of this problem. I am, however, assured that one has to assume for this work a normal distribution of resistances in a random sample of insect test-subjects, otherwise the work cannot be carried out. It should, perhaps, be recorded that Trevan in his work (5) found the frequencies of the occurrence of animals with higher individual lethal doses than the mode fell off less rapidly than that of animals with lower individual lethal doses. I consider it not unlikely that a similar slightly asymmetric distribution might be found among insects as generally sampled for insecticide tests. I hope against hope that I shall be able to find time at some later date to put these tests on a basis that will admit of closer statistical analysis, but it will entail much labour and time. In the meanwhile, one may express regret that H. H. Richardson (8) in his paper, and it is one of considerable importance, has not published his data at all fully, for I believe from the appearance of his curves that the data required for this purpose have, at least partially, already been accumulated by him.

It is as well to admit here, that in doing these tests a random sample is not taken over the whole population of the species of insect test-subjects, owing to the comparatively small number of insects that can be employed conveniently; obviously diseased or parasitised insects are eliminated and an attempt is made either to take them in a late instar or in the adult stage. Every attempt is made to rear them under uniform conditions, although this is not always attainable. Random sampling is really over a rather narrow field. It is thus not surprising that from time to time the test-subjects act almost like one individual. H. H. Richardson found such a case amongst his house-flies. When Gimingham and I (19) were studying the toxicities of the fatty acids, there were indications that Mr Gimingham, over several weeks of

time, reared *Aphis rumicis* of great uniformity of resistance. The results were sufficiently interesting to warrant further work which we could not carry out at the time, and on publishing we deprecated statistical analysis as the 50 per cent. toxic doses had not been determined, only the concentrations giving 100 per cent. of deaths being given. Nevertheless O'Kane, Westgate, Glover and Lowry (7) plotted the reciprocals of these results on a semi-logarithmic scale against the molecular weights on the same diagram as the surface activities. They themselves for the fatty alcohols had determined the concentrations giving 50 per cent. of deaths. The two sets of results were almost identical in significance and they used them, I think quite rightly, to show the important part played in the toxicity of these compounds by adsorption, surface activity and orientation in the surface.

However important it may be to explore the region of the "median" toxic dose, I believe it is necessary to examine the region of the concentrations or of the times

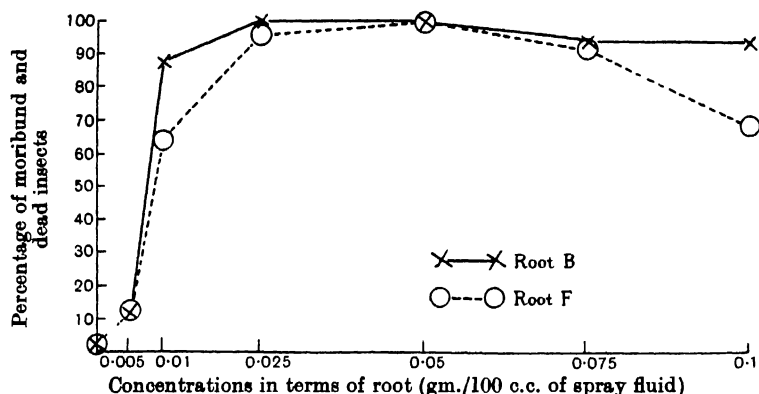


Diagram 3. Toxicity results of two samples of Derris root.  
Test-subject *Myzus persicae*.

giving approximately 100 per cent. deaths. It is of practical importance and if neglected something may be missed.

Diagram 3 gives in graphical form some data for two samples of *Derris* accumulated recently by Mr Newton and me. These slides show figures for one out of three series of tests; all of which gave peculiar results of this type. The two samples had the same content of rotenone, and over a definite range of concentrations (up to 0.05 per cent.) which are expressed in terms of root the results for equivalent concentrations do not differ by more than the experimental error, although sample *F* is always slightly less effective. The results are quite normal up to a certain concentration (0.05 per cent.), and then, above that strength, both samples are shown to be less effective than below it. In the case of root *B*, the results are scarcely significant, but in the case of root *F* definitely significant. Obviously two factors are working in opposite directions. This is not the place for me to give any hypothesis to account for this curious effect. The diagram is given to bring out the point that it is useful to survey a fairly large number of concentrations, and not limit the experiment to too narrow a range.

✓ *The time factor.* The question has frequently been raised as to how far the factor of time should be taken into account in judging toxic effects quantitatively. It is

perhaps necessary to point out that, except in the case of stomach poisons where insects of fairly long life period can be used and for certain experiments in contact insecticides, it would be a matter of considerable difficulty to carry them out. It is probable that a team of workers engaged night and day would be required to make really adequate observations. The question, however, does arise—how should the relative effectiveness of an insecticide be judged? Of the two alternatives: (1) the ratio of the doses required to produce the same effect, say 50 per cent. of deaths, (2) the ratio of the effects produced by equal doses, I consider that the nature of the characteristic curves leaves us no option but to choose the former. The time factor is more subtle and important. Henderson-Smith (12 b), in his paper on the killing of *Botrytis* spores by heat, has pointed out that, in the estimation of temperature coefficients, comparison of the times taken to reach a constant result give more accurate and consistent values than comparisons of the results reached in constant time. How far does this apply to insecticides? H. H. Richardson, in his work (8) on kerosene-pyrethrum extracts, found that when he determined the time required by a constant concentration to paralyse 50 per cent. of his flies the percentage error of his mean was 0.84 per cent., but in determining the numbers of dead after 24 hours it rose to 6.7 per cent. He criticises the latter method as giving widely variable comparisons between different concentrations.

I am personally unable to decide from his data whether the difference in the degree of accuracy is due to the inherent differences in the methods or to some exceptional difficulty in ascertaining the death-point of his flies, or if the time allowed for judging results were long enough. It is true that he was investigating an insecticide not easy to evaluate, having the property of producing deep narcosis which may be fugitive, for reasons that are not yet understood. As a matter of fact, working on bean aphids and determining the numbers of moribund and dead for different concentrations after 48 and 72 hours, we arrived at the same conclusion as Richardson (13) from the observation of the times required to paralyse, namely, that the pyrethrin I content of pyrethrum was correlated with its toxicity. With our insects we have usually found the narcotic effect of pyrethrum so rapid as to render it hardly possible to determine the time required to produce narcosis. It is, too, highly questionable whether this form of technique would give us on the whole information of the type we require. If the rapidity of action had only been taken into account in the past, a number of valuable insecticides would never have come to light. There is a startling difference between the rapidity of effect of the pyrethrins on the one hand and rotenone (the active principles of *Derris*) on the other; for whereas the rapid narcosis produced by the pyrethrins may wear off at concentrations below a certain level, the narcotic effect of rotenone deepens in intensity with time. Thus the time factor in producing narcosis has importance, and I consider we have reached a stage when more stress should be laid upon it. Nevertheless if we are to determine the usage to which an insecticide can be put, the treated insects should be kept a time sufficiently long for one to ascertain what the final effect is going to be. Another important factor, often neglected, is the dosage required to inhibit reproduction (say, of aphides), for there are several compounds capable, below certain levels of concentration, of rendering the adult insect apparently moribund, but which do not paralyse the power of reproduction.

Time does not allow me to enter on a discussion of many other important phases

of this type of work, but it is well to understand that quantitative evaluations, very important though they are, do not give a full and complete compendium of information about insecticides. It may be true that in physics, astronomy and chemistry, science is only concerned with yardsticks; I am not so sure that the principle is true of biology. There seems to me, as one who stands on the fringe of the biological sciences, that there are qualitative properties very difficult to evaluate in terms of percentage concentrations, inches, cubic centimetres and seconds of time. In this small field of applied biological and biochemical work there are ranges of unexplored territory that have not been surveyed. There is the problem of the specific resistance shown by some insects to the effects of certain classes of insecticides; the problem of seasonal variation in the resistance of insects; the problem of the mode of action of insecticides, and so forth. I think it is a fair conclusion to make, that not until some of these questions are dealt with and insect physiology linked on to insect toxicology will our increasing knowledge of the chemistry and physics of spray fluids become a *Materia Medica* for Plant Pests and Diseases completely worthy of the name. The work requires the close collaboration of chemist and biologist.

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II. LABORATORY TESTS OF INSECTICIDES FOR USE  
AGAINST WOOD-BORING INSECTS.

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BEFORE describing the tests which are being carried out with wood preservatives and insecticides at the Forest Products Research Laboratory at Princes Risborough, it should be explained that these tests do not concern forest insects such as longhorn beetles, bark beetles and pinhole borers; they deal solely with two groups of wood-boring insects, namely the powder-post beetles (family *Lyctidae*) which attack certain newly seasoned hardwoods such as oak, ash, walnut, etc. and secondly the furniture beetles (family *Anobiidae*). Belonging to the latter group are the common furniture beetle (*Anobium punctatum*) which attacks old furniture, wicker basket-work, etc. and the death-watch beetle (*Xestobium rufovillosum*) which has been the cause of very extensive damage to the roof timbers of a great many old buildings in this country.

Mention has been made of both wood preservatives and insecticides, and the difference between these should be made clear. The object of a wood preservative is to protect timber against insect and fungal attack. An insecticide, on the other hand, is applied to timber to destroy insects already present in the timber. The work done so far on wood preservatives at the Forest Products Research Laboratory, as far as they affect the wood-boring insects mentioned, comprises a series of tests which form part of a project now in operation at the laboratory for determining the comparative values of the effectiveness of a number of wood preservatives against insect attack and fungal decay. Owing to the difficulties experienced in breeding the furniture beetle and the death-watch beetle, tests with preservatives have been limited to oak sapwood exposed to attack by *Lyctus* powder-post beetles. These tests have consisted of exposing to *Lyctus* attack a series of specimens of oak treated by the full cell process with zinc chloride and another preservative at different concentrations. All the samples contained a proportion of sapwood which alone is attacked by *Lyctus*. Two series of experiments were carried out: (1) in which treated samples and controls,  $6 \times 4 \times 1\frac{1}{4}$  in. were exposed to *Lyctus* attack in specially constructed cages, and (2) in which small sections of sapwood only,  $1\frac{1}{4} \times 1 \times \frac{1}{2}$  in. were cut from the larger standard size samples, and exposed in boxes to *Lyctus*. While the large samples were only examined externally the smaller samples were cut up and examined at frequent intervals to determine the progress of infestation by *Lyctus*. Results obtained indicate that aqueous solutions of zinc chloride in concentrations varying from 0.1 to 2 per cent. prevent neither egg-laying of *Lyctus* nor the hatching of the egg and initial feeding of the larvae. Although, ultimately, larvae feeding in oak treated with the above range of concentrations of zinc chloride died, yet it is of interest to note that when the concentration was 0.2 per cent. some larvae fed for three months before being killed; where the concentration was 2 per cent. it was possible for a larva to feed and survive for approximately one month. In the case of the second salt tested (a proprietary article) although egg-laying was not prevented, by any of the concentrations used (0.05–1 per cent.), fertile eggs did not hatch in samples treated with solutions of concentrations higher than 0.2 per cent.

Even at lower concentrations also, numbers of eggs were destroyed but instances were recorded of larvae emerging and beginning to tunnel in the treated samples. The maximum period during which a larva survived and fed was one month in a sample treated with a solution of concentration only 0.5 per cent.

It is with insecticides, however, that this paper is mainly concerned, and it is to these that most attention has been paid in the control of the two groups of wood-boring insects under consideration, particularly the death-watch beetle. Attention was first called to the extent of damage done by this insect just before the war when the late Prof. Lefroy, in co-operation with H.M. Office of Works, commenced working on insecticides for use against wood-boring insects, having in mind particularly the control of the death-watch beetle in the roof of Westminster Hall. He found that while there are many substances which are toxic to the insect the chief problem to be overcome was that of penetration, and subsequent work has confirmed the fact that one of the essential properties of an insecticide against wood-boring insects is the power to penetrate the wood to as great a depth as possible. This degree of penetration is of special importance when the treatment of timber infested by *Xestobium* is contemplated, as this insect attacks beams of large dimensions and the larvae may be at a depth of several inches within the wood. The greatest difficulty in choosing a suitable insecticide is not that of finding a substance toxic to the insect but of combining toxicity with adequate penetration. The essential properties of such an insecticide (*e.g.* non-inflammable, non-poisonous to man, as permanent as possible, etc.) have been described by Prof. Lefroy in a lecture to the Royal Society of Arts (1).

Soon after death-watch beetle damage was discovered in Westminster Hall, similar depredations were found in old buildings all over the country and the infected timbers were in many cases treated with various proprietary preparations. Several of these, it was claimed, were based upon formulae evolved by Prof. Lefroy, but there is little, if any, information available on the efficacy of these treatments in checking the beetles' ravages. Meantime, investigations into the habits and life histories of wood-boring insects were begun by the Forest Products Research Laboratory and, while it was essential to concentrate on the biological work, the need for determining the relative values of various insecticides became evident. Further instances of damage by death-watch beetle were continually coming to light and enquiries as to methods of treatment naturally arose. In addition, several new substances have come on the market during the past few years and it was desirable to compare these with the earlier preparations. To give an opinion on these substances, however, is a responsibility even when that opinion is based upon scientific tests; it means that one has to recommend the preparation or alternatively show that it is useless, but when insufficient is known of the life cycle and habits of the insects with which one is dealing the tests under such conditions are of very little value. To carry out really satisfactory tests with insecticides against wood-boring insects it would be necessary to have timber of a known standard of infection, but until more is known of the biology of *Anobium* and *Xestobium* such material cannot be acquired. Nevertheless, it was found necessary to carry out certain preliminary tests to ascertain the relative value of insecticides pending the acquisition of larger stocks of *Xestobium* and *Anobium* and a wider knowledge of their habits and life history which in time will enable more satisfactory tests to be conducted. Little information could be obtained from previous work, except from the results of a number of preliminary tests carried

out by Dallimore at the Royal Botanical Gardens, Kew, on behalf of H.M. Office of Works, and Bolle's work in Germany (2).

Even the preliminary tests that have been conducted have proved unsatisfactory. In the first place, it is often very difficult to determine whether or not a certain sample of wood contains living insects, especially when dealing with timber attacked by the death-watch beetle. The presence of piles of fresh bore dust, expelled by the larvae working within the wood is a certain guide with *Anobium* and *Lyctus* that the wood contains living larvae. The larvae of the death-watch beetle, however, rarely expel the dust from their tunnels and from the large quantity of infected timber which has been collected at Princes Risborough showing extensive damage by these insects, it is not possible to determine whether a piece of timber does or does not contain living insects. The condition of the wood and the extent of attack may be some slight guide, but the only certain method of determining whether or not the timber contains living insects is to cut up the sample of wood in question. The use of X-rays has been tried in detecting the presence of living insects within wood, but the results obtained were not satisfactory and the method has not been developed further. When tests were first started, samples of wood attacked by death-watch beetle were included, but subsequent examination showed that these were so often deficient in living insects that the tests were useless and this material was not included in later tests. At other times it was found that while the treated samples contained sufficient insects to draw some conclusions from the experiment, so few living larvae were present in the untreated control samples as to make the tests in question valueless. It was found that the material that could be relied upon to contain an average number of living larvae was oak attacked by *Lyctus* powder-post beetles and sycamore attacked by *Ptilinus pectinicornis*, a beetle belonging to the family Anobiidae, but not of such economic importance as *Anobium* or *Xestobium*. Both *Lyctus* and *Ptilinus* material, however, have their disadvantages. *Lyctus* attacks only the sapwood, so that tests with this insect provide no data upon the powers of penetration in heartwood which is so important in the case of *Xestobium*. The damage caused by *Ptilinus* is not truly comparable with that due to *Xestobium*, and furthermore, this insect usually confines its attack to sycamore or beech, timbers rarely infested by the death-watch beetle.

The tests that have so far been carried out have consisted of applying insecticides with a brush to samples of infected wood, measuring approximately  $4 \times 2 \times 2$  in. Alternatively, a small hand sprayer has been used, but when treating only small samples of wood it was found that no better results were obtained with this. When treating large timbers *in situ* in a building, however, the application of the insecticide by means of a sprayer is both quicker and more satisfactory for reaching cracks and crevices in the wood. Samples of oak attacked by *Lyctus* were selected from material showing fresh bore dust. As this insect attacks only the sapwood, samples were chosen containing not less than 50 per cent. infected sapwood giving a possible penetration of about  $\frac{1}{2}$  in. for the insecticide. Care was taken not to choose material which was in a too powdery condition. Sycamore samples attacked by *Ptilinus* and containing both sapwood and heartwood were also selected from material showing larval activity.

The insecticides tested have consisted of twelve proprietary preparations including many well known brands, but tests have also been carried out with two



samples of chlorinated phenols and a range of low temperature tar phenols (supplied by the Chemical Research Laboratory at Teddington) and also with orthodichlorobenzene. The choice of a suitable standard insecticide for comparative purposes has been a difficulty, but meanwhile this has been overcome by the adoption of orthodichlorobenzene as the standard in the tests so far carried out. With most of the insecticides that have been tested twelve samples of oak attacked by *Lyctus* were treated and the same number of sycamore attacked by *Ptilinus*. Six of the samples of each of these two groups are given one treatment, while six receive a second coat one week after the first. Treated samples are cut up for examination within a few weeks of treatment, though since every splinter of wood has to be examined for larvae the examination is necessarily a very lengthy and tedious one. Care is taken to note as accurately as possible at what depth from the nearest surface the insects, dead or alive, are found. Untreated control samples are cut up at the same time. Difficulties are sometimes experienced in differentiating between larvae that have been killed by treatment and those killed by certain parasitic and predaceous insects which are frequently found invading infected timber.

In addition to the tests already described, the effects of the relative toxicity of the fumes given off by various insecticides have been tried on larvae removed from the wood. The larvae are suspended in muslin inside a specimen tube in the bottom of which, but not in contact with the larvae, are about 5 c.c. of the insecticide under test. They are examined at intervals of 24 hours, and larvae under control conditions are also inspected at similar intervals. Difficulties are experienced in determining whether larvae have been killed or not. Methods employed in detecting the likelihood of the recovery of larvae are the same as those given by Hamlin and Reed, two American entomologists, in a paper describing the effects of fumigation with carbon bisulphide on the larvae of the Indian meal moth (*Plodia interpunctella*) (3). Larvae which have been exposed to insecticide fumes are characterised by varying degrees of shrivelling, limpness and discoloration, but a more definite method of determining whether larvae are really dead is needed.

No work has yet been done on fumigants, though the need for such work is apparent when the difficulties are realised of obtaining adequate penetration of timber by a liquid insecticide.

Finally, methods of tests described in this paper serve to show the unsatisfactory nature of testing insecticides against wood-boring insects. The future development of accurate and reliable laboratory tests depends not only on the production of material of an even standard of infection, but also upon information on the penetration of liquids and gases into timber and upon chemical studies of insecticides suitable for the control of wood-boring insects.

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## REVIEWS

*Insect Pests of Farm, Garden and Orchard.* By E. DWIGHT SANDERSON, Ph.D. Third edition, revised and enlarged by LEONARD MARION PEAIRS, Ph.D. Pp. viii + 568, with 607 figures. New York: John Wiley and Sons, Inc.; London: Chapman and Hall, Limited, 1931. Price 31s. net.

Over ten years have lapsed since the second edition of this standard work. During this time great progress has been made in the knowledge of the biology of insects and the methods of dealing with such pests. Parallel to these advances there has been a corresponding outpouring of text-books on the subject, some good, some only mediocre. Besides this, applied entomology may be said to have reached the cross-roads. It is not sufficient to be able to control an outbreak of a pest when it has occurred and indeed it would be better to leave this to the chemists. It is essential that workers shall realise that prevention is better than cure and that the fields of epidemiology and ecology offer the most promising ground for investigation. Furthermore, applied entomology can no longer ignore plant pathology, neither can plant pathology stand aloof from applied entomology. Such being the case, it is perhaps pertinent to ask whether a third edition of a book, necessarily based very extensively on the older idea of recognising the pest and then killing it, is desirable.

Turning to the book in question, one is at once struck by the careless way in which it is written. On p. 19 we find this sentence—"Following a meadow grass crop which has permitted the development of considerable numbers of root-feeding forms which, on account of the numbers of individual plants, did no great damage, with some one of the grass plants, also suitable for the insect but planted in hills, gives opportunity for the insects which survive the cultivation to concentrate their damage on a relatively small number of plants with consequent severe injury." Again, on p. 22 there is—"When modification of cultural practices, in the broad sense, shall have been made, without extra cost to the farmer and without decreasing his yields or interfering with his system of farming, which shall have reduced insect damage to the point where it is no longer of economic importance, that is, no longer measurable, the problem may be considered solved." And yet again, on p. 35 there are these statements—"We have chosen to include here the use of heat and of cold—physical agencies rather than chemical—purely for purposes of convenience. *This procedure may be justified by the fact that we use hot air and cold air in most cases where temperature is involved. Hot air may properly be classified as a chemical and a fumigant.*" The italics are our own.

Other indications of the apparent haste in which the book was compiled are as follows. The legend for Fig. 19 continues a mistake that was made in the legend of the original figure in *U.S.D.A. Bull.* 276 (1915) but which was later emended by a correction slip. It should read "Larva of the Cecidomyid fly *Aphidoletes* sp., which preys upon *Macrosiphum pisi*. Enlarged." This figure was published with the correct legend in an article by J. J. Davis (*Journ. Agric. Res.* vi, 1916, 883-8). Figs. 29 and 83 seem to be identical. On p. 177 the clover head caterpillar is placed in the genus *Laspeyresia* in one place and in *Enarmonia* a few lines later. The same thing occurs on pp. 400 and 401 where the Terrapin Scale is first called *Lecanium* and later *Eulecanium*. Fig. 343 is spoilt by two black smudges which do not appear in the original illustration. The wheat midge is called *Contarinia tritici*, although Felt as far back as 1912 stated that no American species could be referred to *C. tritici*. The name should be *Sitodiplosis mosellana* and indeed the illustrations given in Fig. 52 confirm this view. It seems rather odd also to find *Pontia* being used instead of *Pieris* in dealing with *P. rapae* and *napi*.

Apart from such errors as these, the book contains a vast amount of new and up-to-date information. Since the previous edition it has been very largely rewritten and now the data is set before one in a rather more concise manner, perhaps even dogmatic in certain cases. In this way it has been possible to add to the book without increasing the number of pages. The illustrations have for the most part been well chosen and in some instances are very good.

At the beginning of the volume there are chapters on the injury to crops by insect pests and on the structure and development of insects. Next come four chapters on insect control: general, cultural and mechanical; biological; chemical; and methods and apparatus. Subsequently the insect pests of farm, garden and orchard crops receive detailed attention. In addition, there are chapters on insects affecting stored products, livestock, poultry and man. The section on bibliography of the previous edition has now been omitted, references being inserted in the context. Finally there is an adequate index.

H. F. BARNES.

*Handbuch der Pflanzenkrankheiten.* By Dr L. REH. Fünfter Band. Tierische Schädlinge an Nutzpflanze. Zweiter Teil. Pp. 1032, with 468 text-figs. RM 84. Berlin: Paul Parey, 1931.

Sorauer's *Handbuch der Pflanzenkrankheiten* first appeared in 1879 but it was not until 1913, when the work was in its third edition, that a volume on the "Animal Enemies" of plants made its appearance under the authorship of Dr Reh of Hamburg. This latter volume may be regarded as the natural successor to the *Tierische Schädlinge* of Ritzema-Bos (1891), and, like it, has become a classic known to entomologists throughout the world. Even in 1913 it was a stupendous task to summarise the scientific knowledge then existing over so wide a field, but no words can properly describe the task of revising such a work in the light of the knowledge of the present day. Some idea may perhaps be gained from the fact that the 1913 volume occupied 774 pages, whereas the revision now completed in two volumes fills 1515 pages. The exclamation "Kolossal" seems barely adequate!

The volume actually under review, the second of the revised edition, deals with the Insect Orders Diptera, Coleoptera, Hymenoptera, Rhynchota (in all 750 pages), and with the Vertebrata (200 pages), these sections being followed by a general account of methods of control and an index, which latter alone occupies 37 pages. This brief description of the contents will be sufficient to show that the work is not one which a reviewer can read from cover to cover: the most he can do is to test the contents here and there and form his opinion on the sample. Judged in this manner, the revision has on the whole been well done, and as compared with previous editions it is notable that a better balance has been retained between the amount of space devoted to West European pests and to those from the remainder of the world. As is perhaps inevitable in a work which has taken some years to prepare, the different sections are not all equally up to date, and in this respect the section dealing with the Diptera—the first in the book—is not so satisfactory as the succeeding sections. No reference, for instance, is made to *Eumerus tuberculatus* Rond., which is now generally recognised as the most abundant and destructive of the Small Narcissus Flies, and the account of the Wheat Bulb Fly states the position as it was in 1918. No doubt it is still true to say of this latter species (as of many others) that the biology is "noch recht ungenügend bekannt": but our knowledge has been greatly increased in the past ten years, and some later references might have been expected.

In general, however, there is little to complain of in the scope and character of the information provided, which is in all cases well supported by references: every reader will no doubt be able to point to omissions, notably in references to work within his own special field, but criticisms on this score would be ungracious since the inclusion of everything is manifestly impossible and the references are in the

majority of cases ample, both as authority for the statements made in the text and also as a guide for the student in search of further knowledge.

The nomenclature adopted is ordinarily that which is familiar to the economic entomologist, but in this respect the section on Aphidina is to some extent an exception, and whatever may be the verdict of the systematist a feeling of dismay may be expressed at the appearance of the Rosy Apple Aphis under yet another alias, *Yezabura malifolii* Fitch. We had hoped that in *Anuraphis roseus* Baker this unfortunate insect had at last achieved a name with some claims to permanence!

The text figures are well chosen and usually good, sufficiently so, indeed, to justify the use of the somewhat heavy paper on which the book is printed, and which results in the total weight of 5½ lbs. for the volume!

Summarising the impressions gained from the book as a whole, the veteran author, Dr Reh, and his colleagues, twelve in number, are to be congratulated on the successful achievement of their task. Their work will stand as a monument to the knowledge of the plant pests of the world which was in the possession of mankind during the first quarter of the twentieth century. In addition, it may not improbably prove to be the last work of this type and scope which will ever be written, for knowledge is increasing at such a rate that it is almost unthinkable that any author, or collection of authors, should again face the task of writing a text-book on the injurious animals of the world! In his preface Dr Reh says, apparently not without some regret, that he has devoted twenty-six years of his life to *Sorauer*, neglecting his own special interests and other work on its behalf. We can assure him that these twenty-six years have not been wasted, and that he can now take a well-earned rest in the satisfaction of work well done.

J. C. FRYER.

*Chemical Embryology*. By Dr JOSEPH NEEDHAM. In 3 volumes. Vol. I, pp. xxii + 613 + 11 plates. Vol. II, pp. xvi + 615-1253 + 3 plates. Vol. III, pp. xvi + 1255-2021 + 1 plate. Cambridge University Press, 1931. 105s. net.

This book does not come within any strict definition of Applied Biology, but there can be few biologists of any kind who will not find in it something to interest them. The title is a little forbidding and may cause some not versed in chemistry to hesitate before delving into three massive volumes containing over 2000 pages. The author regards himself as the "obstetrician" of a new and independent science, "Chemical Embryology," but many biologists will think that this is simply "the general and developmental physiology of the embryo" writ large.

A summary of the contents will give the measure of the work which is planned in five parts, the latter in no way coinciding with the separate volumes. Part I—The Theory of Chemical Embryology—is a short and interesting discussion of biological philosophy leading up to neo-mechanism. Part II—The Origins of Chemical Embryology—comprises some 200 pages and is a finely written and very scholarly history of the subject. It is arranged in three sections, Embryology in antiquity, from Galen to the Renaissance, and in the seventeenth and eighteenth centuries. Part III—General Chemical Embryology—is the main part of the work and extends to some 1400 pages containing 24 sections: (1) The unfertilised egg as a physico-chemical system; (2) Increase in size and weight; (3) Increase in complexity and organisation (one of the most interesting chapters in the book); (4) Respiration and heat production of the embryo; (5) Biophysical phenomena in ontogenesis; (6) General metabolism of the embryo; (7) Energetics and energy sources of embryonic development; (8) Carbohydrate metabolism; (9) Protein metabolism; (10) Metabolism of nucleins and nitrogenous extractives; (11) Fat metabolism; (12) Metabolism of lipoids, sterols, cycloases, phosphorus and sulphur; (13) Inorganic metabolism; (14) Enzymes in ontogenesis; (15) Hormones in ontogenesis; (16) Vitamins in ontogenesis; (17) Pig-

ments in ontogenesis; (18) Resistance and susceptibility in embryonic life; (19) Serology and immunology in embryonic life; (20) Biochemistry of the placenta; (21) Biochemistry of the placental barrier; (22) Biochemistry of the Amniotic and Allantoic liquids; (23) Blood and tissue chemistry of the embryo; (24) Hatching and birth. Included also in Part III are 46 pages of Epilegomena which form the most generally interesting part of the work: in fact when one reaches this portion it is very difficult to put Vol. III down. Part IV consists of four appendices, two of these being accounts of "The Chemical Changes during the Metamorphosis of Insects" written by Dorothy Needham and "The Development of the Plant Embryo from a Physico-chemical Viewpoint" by Muriel Robinson. To a botanist the latter will seem out of place in this work. Part V contains a Bibliography of over 7000 references, at the end of which the author modestly cites an additional 83 which he has not been able to find in England and has therefore not consulted. Two of these are in the Rothamsted Library. There follow 42 pages of Subject Index and an Index Animalium containing the names of some 900 animals. The work is illustrated by 15 plates, 4 charts, 532 text-figures and 293 numbered tables, many of the latter extending over several pages or being printed on enormous folders. There are also in the text many unnumbered tables.

It is not possible to review, in any ordinary sense, this epic work. The treatment throughout is extremely detailed and complete and the whole is written in so scholarly a manner and with so broad and philosophical an outlook that one reads on hardly conscious of the vast amount of information contained in the work or the years of industrious labour that must have been involved in its production. In view of the latter the up-to-dateness of the work is remarkable, and as an example may be mentioned the reference in the text of p. 1626 to the isolation in crystalline form of the anti-rachitic vitamin D. Although necessarily largely a compilation, the methods, data and viewpoints of previous workers are thoroughly digested and resynthesised by the author, and the book bristles with original thought and stimulating and constructive criticism. On p. 553 the author protests that "The purpose of this book is to give all the facts that are known about the physico-chemical aspects of embryonic development, and not the theories, which indeed, would demand a much larger treatise," but one refuses to believe that the author has not taken delight in his iconoclasm. Although here and there just a little ponderous and heavy with classical lore, there is a freshness about the book that is delightful—how many authors could insert a stanza from Spenser's *Faerie Queene* in the midst of a highly technical consideration of the unfertilised egg as a physico-chemical system and leave one with the feeling that it was just the right thing to do? In the preparation of his book the author has taken as his models two of the greatest biological works in English published in the present century—Marshall's *Physiology of Reproduction* and d'Arcy Thompson's *Growth and Form*, and his book is worthy to stand with these masterpieces. Whether or not Dr Needham has justified his case of "delivering" a new and autonomous scientific discipline only time will show, but there can be no doubt that he has written a book that will place his name high in the very honourable record of English biological science. Praise must be given to the Cambridge University Press for the beautiful way in which the *magnum opus* has been produced.

WILLIAM B. BRIERLEY.

*Common Pests. How to Control Some of the Pests that Affect Man's Health, Happiness and Welfare.* By RENNIE W. DOANE. Pp. viii + 398, with 215 figures. Size  $5\frac{1}{4} \times 8\frac{1}{4}$ . Springfield, Illinois: Charles C. Thomas; London: Baillière, Tindall and Cox. Price 21s. net.

*Common Pests* is a welcome change from the massive text-books on injurious insects that have recently been published. It is a small neat volume that would adorn any bookshelf. Written with authority in a semi-popular vein, it should attract many general readers. Naturally it deals essentially with the American fauna.

There are two sections. In the first, "Pests of Man and Domestic Animals," eight chapters deal with some near relatives of insects; blood-sucking flies; mosquitoes and their control; mosquitoes and disease; flesh flies, screw-worm flies and bot flies; house flies and disease; bedbugs, lice and fleas; and finally parasitic worms.

The second section, "Insect Control and Some Important Pests of the Orchard, Garden, Field and Household," covers a still wider field. Starting with the control of insect pests, there are chapters on insect pests of the orchard, on citrus pests, on insect pests of berries, grapes, vegetable and truck crops, of field crops and of shade and forest trees including those in lumber. Then pests of flower gardens, greenhouses, mills, storerooms and houses receive attention. Finally there are two chapters on mammals and birds as pests. To conclude the book there is an adequate index of thirteen pages.

In spite of the wide ground it covers, the book is not at all unwieldy. The information contained is concise, to the point and up-to-date. The plentiful illustrations are excellent. The general format is very attractive. We have no hesitation in recommending the book as a handy reference book both for scientists and the general public.

H. F. BARNES.

*A Text-Book of Experimental Cytology.* By J. GRAY, M.A., F.R.S.  
Pp. x + 516, 4 plates. Cambridge University Press, 1931. 25s. net.

This volume contains the substance of a series of lectures delivered by the author in Cambridge for some years past and represents the impressions which he "as a biologist, has gained by contact with such physical facts as appear to him to bear on the structure of living matter, and which can be imparted to others like himself, whose knowledge of inanimate matter is limited."

The scope of the work is shown by the chapter headings, which are as follows: I, The cell as a unit of life; II, The cell as a physical unit; III, Cell dynamics; IV, The cell as a colloidal system; V, The physical state of protoplasm; VI, Cell membranes and intercellular matrices; VII, The nucleus; VIII, Mitosis; IX, Cell division; X, The shape of cells; XI, The growth of cells; XII, Cell variability; XIII, The equilibrium between a living cell and water; XIV, The permeability of the cell surface; XV, The nature of the cell surface; XVI, The germ cells; XVII, Contractile cells; XVIII, Phagocytosis. Each chapter is followed by a useful bibliography and the book closes with a somewhat inadequate subject index and an author index containing some 400 names. There are 205 text-figures, four plates (one coloured) and 75 tables. "Arrhenatherum" is spelled wrongly on pp. 120, 121 and in the Index, there are misprints in line 24, p. 296 and in the legend of Fig. 9 B, and a slip of type in Table XIX. Also one could wish that Mr Gray had not adopted throughout (save in Table XII) the spelling "Paramecium."

In many ways animal cytology has made far more progress than plant cytology. The former is as much physiological as morphological and embraces the study of the structure and functioning of the entire cell in relation to its biological and physical environment. The latter is almost purely morphological, with vision bounded by the staining technique, and it has remained largely the study of the cell nucleus and particularly the chromosome apparatus. There is urgent need for botanical cytologists to become more physiologically minded and to see plant cells not merely as so much extraneous matter impeding the chromosome view, but as living differentiating "units" of functional activity, integrating with each other in a complex and ever changing biological and physico-chemical environment. It may be true, as the author says, "it looks as though the fundamental structure of the cell is of an order quite remote from that revealed by microscopic methods," and one cannot help feeling that, in a way, it is almost more important that the present book should come into the hands of botanical rather than zoological students. The volume is essentially a treatise on the experimental physiology of the cell concerning itself with the applica-

tion of physico-chemical methods of investigation and interpretation to the study of living cells. The material is presented from the mechanistic standpoint, but the mechanistic explanation is nowhere pressed beyond what is reasonable and its difficulties and uncertainties are always pointed out. As the author says: "Any attempt to analyse the relationship of the organism to its external environment without an accurate and precise knowledge of physico-chemical laws is doomed to failure," but again "a biological problem disguised by the sparkling terminology of the chemist is too often a pathetic and rather disreputable object."

Mr Gray has performed a difficult task well and has brought together in a very useful and readable way a great number of widely scattered data bearing on his theme. Botanists, particularly, will welcome the able manner in which the brilliant work of Osterhout and his collaborators has been incorporated into the author's picture. Mr Gray writes with that insight and intimate knowledge which only personal investigation can give, and one feels that he has carefully selected and thoroughly digested his material. Throughout, his book is noteworthy for its impartiality and its careful and temperate criticism and it must certainly be regarded as an outstanding contribution to recent biological literature. The format, beautiful printing and illustrations of the book maintain the very high standard adopted by the Cambridge Press.

WILLIAM B. BRIERLEY.

## REPORT OF THE COUNCIL OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS FOR THE YEAR 1931

DURING 1931 the Association has met on five occasions, including one field meeting.

This was held at the Farnham House Laboratory of the Imperial Institute of Entomology, and the Association is indebted to Sir Guy Marshall, Director of the Institute and to Dr Thompson, Superintendent of the Laboratory, for their hospitality.

The attendance at meetings has been on the average 60 per meeting, which is higher than in previous years, and discussion of papers has been more active. This, it may be presumed, justifies the reduction in the number of meetings which was tentative.

During the year, the Association has lost ten members through resignation, and the Council have, with regret, to record the death of Dr T. F. Chipp, a former Secretary of the Association, of Dr Stenhouse Williams, a former Member of Council, and of Dr F. A. G. Muir.

The number of new members elected during the year was fifteen, which gives an increase of membership of two. The Association now numbers 291 ordinary members and 12 honorary members.

During the past year the Association has again enjoyed the privilege of holding its meetings in the Botanical Department of the Imperial College of Science and Technology, and the Council takes this opportunity of recording its grateful thanks on behalf of the Association to the College authorities for this valued hospitality.

The following papers and discussions were brought before the Association during the year 1931.

*Feb. 20th.* Messrs BRIERLEY, BRUCE-WHITE, GOODEY, THORPE and TURRILL: "Biological Races and their Significance in Evolution."

*Mar. 20th.* (1) Dr A. C. THAYSEN: "Retrospects and Prospects of the Economic Application of Microbiology." (2) Mr H. S. BUNKER: "Microbiology of Cellulose."

*Oct. 30th.* Mr R. H. STOUGHTON: "Nuclei, Reproductive Bodies and Cell Fusion in a Bacterium." Mr T. H. TAYLOR: "Control of Levuana Moth in Fiji." Dr P. H. GREGORY: "The Fusarium Bulb Rot of Narcissus." Messrs BRIERLEY and MUNRO: "The Training of Biologists for Economic Posts."

*Dec. 4th.* Messrs FINDLAY and MARTIN: "Laboratory Tests of Fungicides."



## REPORT OF THE HON. TREASURER FOR THE YEAR ENDING DECEMBER 31, 1931

During the year ending December 31st, 1931, subscriptions and entrance fees amounting to £339. 12s. 11d. were received from members. This sum represents a decrease of £6. 14s. 9d. as compared with the previous year. It is more than counter-balanced by an increase of £35. 0s. 2d. in the amount of the sales of the *Annals of Applied Biology* to non-members; and is largely due to the fact that a number of members are in arrears with their subscriptions. It is requested that all members who are still debtors to the Association will discharge their obligations as promptly as possible. It is also very desirable that the membership of the Association shall be enlarged, and members are requested to point out to possible subscribers the advantages of belonging to the Association.

The income for the year exceeded expenditure by £202. 14s. 2d. After all obligations are met the assets of the Association amount to £947. 12s. 2d., of which £806. 5s. 0d. is represented by National Savings Certificates.

J. HENDERSON SMITH,  
*Hon. Treasurer.*

# THE ASSOCIATION OF ECONOMIC BIOLOGISTS.

Dr. ANNALS OF APPLIED BIOLOGY INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED DECEMBER 31st, 1931. Cr.

EXPENDITURE.		INCOME.	
£	s. d.	£	s. d.
To Estimated Value of Stock at January 1st, 1931.	48 13 1	By Sales—Current Volume and Parts	594 19 4
To Cambridge University Press	1038 15 9	By Sales—Back Volumes, Parts and Sets	98 10 11
To Copies Bought in	17 8 9	By Sales of Reprints	189 15 0
		By Contributions to cost of papers, etc.	48 18 9
		By Cambridge University Press advertisement	1 13 9
		By Estimated Value of Stock at December 31st, 1931	39 8 6
		By Balance carried down	131 11 4
			<u>£1104 17 7</u>

Dr. GENERAL INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED DECEMBER 31st, 1931. Cr.

EXPENDITURE.		INCOME.	
£	s. d.	£	s. d.
To Annals of Applied Biology, balance brought down	131 11 4	By Members' Subscriptions:	18 2 0
To Printing and Stationery	7 15 8	Arrears	9 9 0
To Postages and Cheque Stamps	6 6 3	Entrance Fees	312 1 11
To Honorariums	12 15 0	Current	
To Sundry Out-of-Pocket Expenses of Secretaries and Treasurer	5 11 9	By Interest on National Savings Certificates and Bank Deposit	
To Audit Fee Reserve	4 4 0		
To Balance, being Excess of Income over Expenditure for the year	202 14 2		
	<u>£370 18 2</u>		<u>£370 18 2</u>

## BALANCE SHEET, DECEMBER 31st, 1931.

LIABILITIES AND SURPLUS.		ASSETS.	
£	s. d.	£	s. d.
Sundry Creditors:		Cash:	
Cambridge University Press	171 5 6	At Bank on Current Account	99 2 8
Audit Fee Reserve	4 4 0	At Bank on Deposit Account	350 0 0
Subscriptions paid in advance	175 9 6	Debtors for Subscriptions 2 years or less in arrear	449 2 8
Excess of Assets over Liabilities:		and considered good	
As Balance Sheet of December 31st, 1930	744 18 6	500 National Savings Certificates	51 5 0
Add: Balance of Income and Expenditure Account for 1931	202 14 2	Stock of Annals of Applied Biology at estimated value	606 5 0
			39 8 6
			<u>£1146 1 2</u>

J. HENDERSON SMITH, *Honorary Treasurer.*

We certify that the foregoing Accounts are properly drawn up in accordance with the books, vouchers and documents produced to us, and, in our opinion, the Balance Sheet exhibits a true and correct view of the state of the affairs of the Association.

H. J. COX & CO.  
Incorporated Accountants. Auditors.

HARPENDEN, February 1932.



STUDIES ON PLANT VIRUS DISEASES<sup>1</sup>XI. FURTHER EXPERIMENTS WITH A RINGSPOT VIRUS:  
ITS IDENTIFICATION WITH SPOTTED WILT  
OF THE TOMATO

By KENNETH M. SMITH, D.Sc., Ph.D.

(Potato Virus Research Institute, School of Agriculture,  
University of Cambridge.)

(With Plates XIV—XVIII and 7 Text-figures.)

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## I. INTRODUCTION.

IN 1929 there was discovered in a commercial glasshouse in Cardiff a virus disease affecting the ornamental plant *Solanum capsicastrum*. The symptom expression took the form of numbers of concentric rings upon the leaves; this disease was studied by the writer and a preliminary

<sup>1</sup> The title of this series of papers has been slightly altered to allow the inclusion of studies upon virus diseases other than those specific to the potato plant: No. X in the series appeared in *Proc. Roy. Soc. B*, CIX, 1931.

account published<sup>(16)</sup>. The purpose of the present paper is to describe further studies on this virus and to offer evidence of its identification with the serious disease of tomatoes which is known in Australia as "spotted wilt"<sup>(14)</sup>.

The writer is indebted to Mr J. P. Doncaster for his assistance, especially for making the drawings of the insects and for taking most of the photographs. Acknowledgment is also due to Miss E. McGill and Dr G. D. Morison for identifying the Thrips used in these experiments; Dr Morison has kindly assisted in other matters connected with studies on the Thrips.

After this work had been completed a paper by Bald and Samuel appeared (*Council Sci. Indust. Res. Australia*, Bull. 54, 1931) in which these workers described results very similar to those published in the present paper. In the writer's opinion the similarity of the results achieved in the two independent studies is very strong evidence that the same virus is under consideration in both cases.

## II. INSECT TRANSMISSION STUDIES.

### (a) *Thrips tabaci* Lind. as a vector of the ringspot virus.

In a previous paper<sup>(16)</sup> the writer described some preliminary insect transmission studies of the ringspot virus, using as potential vectors the aphides *Macrosiphum gei* Koch and *Myzus persicae* Sulz. and the insect *Thrips tabaci* Lind. The results achieved appeared to indicate that while *Myzus persicae* could act as an occasional and inefficient vector, *Macrosiphum gei* and *Thrips tabaci* were unable to transmit the virus. Further extensive trials with *T. tabaci* have not borne out this view, and evidence will be presented which indicates that the Thrips is a most efficient vector of this disease (see Table I).

Samuel and Bald<sup>(13)</sup>, working with the Thrips *Frankliniella insularis* and spotted wilt of the tomato, and Linford<sup>(9)</sup>, with *Thrips tabaci* and yellow spot of pineapple, have shown in each case that the Thrips cannot pick up the virus *de novo* in the adult state, it is necessary for the insect to feed upon the diseased plant as a larva in order to become infective. The writer has found this to be the case also with *T. tabaci* and the present ringspot virus, which will be shown later to be the same as tomato spotted wilt. The negative results of the writer's preliminary attempts to induce *T. tabaci* to transmit this virus are due therefore to this curious phenomenon as only adult Thrips were used in the experiments. It must be admitted that this discovery throws some doubt on the validity of

the results achieved by the writer in his apparently successful transmission of the virus by the aphid *Myzus persicae* to certain plants. It cannot definitely be said that these experiments were carried out under conditions which excluded the possible entrance of some infective Thrips. At the moment therefore in the absence of further confirmation the writer's previous statement that *M. persicae* occasionally transmits this virus must be regarded as unproven.

In Table I are given details of a number of successful transmission experiments with *Thrips tabaci* and the ringspot virus upon various plant hosts.

Table 1.

*Experimental transmission of the ringspot virus by means of Thrips tabaci; 6 plants used in each experiment.*

No. of exp.	Source of ringspot infection	Species of experimental plant used	No. of plants infected	Incubation period in plant (days)
1	<i>Datura stramonium</i>	Tobacco (White Burley)	4	10-12
2	"	"	5	10-14
3	"	Petunia	4	2-4*
4	"	<i>Datura stramonium</i>	3	10-14
5	"	"	5	10-16
6	"	Aster	3	14-21
7	"	Tomato	6	10-16
8	Tomato	"	4	12-21
9	<i>Datura stramonium</i>	Potato	4	14-16
10	"	Plantain	4	14
11	"	Zinnia	2	20-21

\* Local lesions, no systemic infection.

(b) *Technique and methods of Thrips management.*

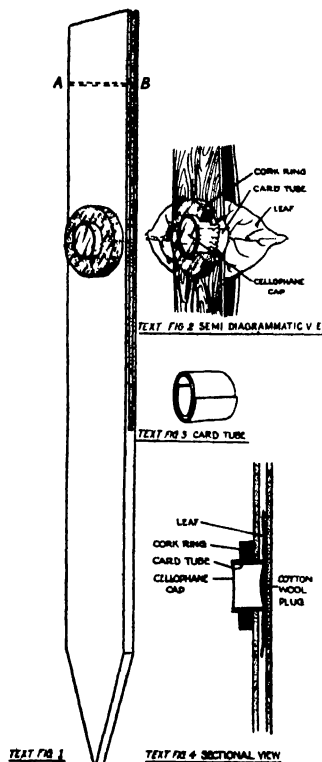
A good deal of difficulty has been experienced in developing an efficient technique for handling and controlling such a small insect as *Thrips tabaci*. Various kinds of insect cages have been used including Storey's "small tube technique" (22) and the modification of this used by Samuel, Bald and Pittman (14); glass lamp chimneys plugged with cotton-wool and adjusted over a leaf have also been tried. None of these methods, however, have proved satisfactory under the writer's conditions. In the case of the tube and spring-clip method, owing to the difficulty of adjusting the pressure of the spring correctly, either the Thrips escaped or the part of the leaf covered by the tube was killed. As regards the lamp chimney plugged with cotton-wool, the chief drawbacks to this method were firstly the loss of Thrips due to drowning in the moisture condensing inside the globe and secondly the dispersal of the insects within the cotton-wool plug. The apparatus which was finally used and which proved satisfactory was a modification of Floyd Smith's

micro-cage (15). Floyd Smith devised this cage chiefly for leafhoppers, and it was found that, as originally designed, it was not suitable for Thrips. The cage was therefore modified in certain points and the apparatus, as finally used, is illustrated in Text-figs. 1-4. The cage consists of a strip of white pine, 12 in.  $\times$  1 in.  $\times$   $\frac{3}{8}$  in., and pointed at one end for insertion in the soil of the pot; in the case of small seedlings, however, the apparatus is placed horizontally across the top of the pot.

There is a saw cut, 8 in. long and  $\frac{3}{8}$  in. wide, parallel to the flat surface. This divides 8 in. of the strip into two parallel portions each  $\frac{1}{8}$  in. thick, and through one of these a hole  $\frac{7}{8}$  in. in diameter is bored, 4 in. from the end. In this is inserted a short cardboard tube covered with a cellophane cap; a glass tube may be substituted for the card tube, this allows of clearer vision but the humidity is apt to become too great for larval Thrips. The cellophane cap should be moistened before it is put in place and should be allowed to dry in position; a cork ring is then glued round the card tube thus holding it firmly (see Text-fig. 2). The leaf of the plant is inserted between the split halves of the wooden strip and the base of the card or glass tube brought into contact with the leaf. A pad of cotton-wool is next placed under the leaf in the manner shown in Text-fig. 4 in order to make close contact between the leaf surface and the base of the tube. The Thrips are introduced into the card

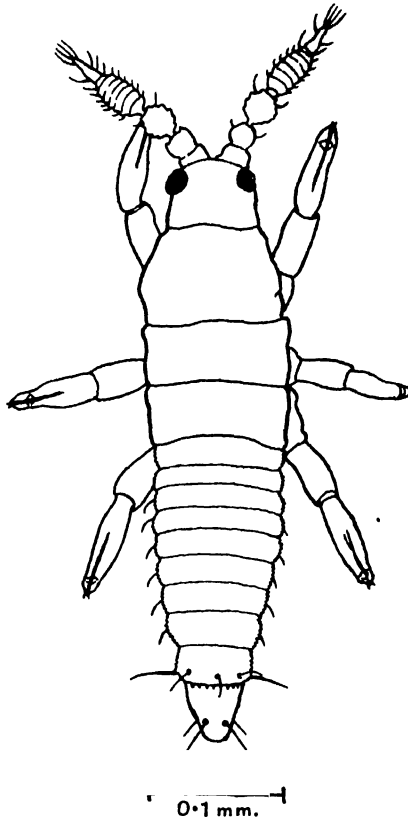
tube and the cage is closed by adjusting the elastic band A-B (see Text-fig. 1). In order to make the cage quite secure it was found necessary to keep the tube of such a diameter as would fit between the larger veins of the *Datura* or tobacco leaf, as if the base of the tube traversed a vein, young larval Thrips were liable to escape at that point. The use of this cage allows close observation to be kept on the Thrips, and the insect is not lost sight of during the process of pupation.

Large cages consisting of metal supports covered with cellophane were also used for breeding Thrips. These cages were placed over a potted plant standing in a deep saucer filled with sand.



(c) *Description of Thrips tabaci* Lind. (adult female).

The following description of the adult female of *Thrips tabaci* is supplied by Mr J. P. Doncaster, who is making a study of the insect; these data are based on his own observations assisted by the descriptions of Morison (*in litt.*) and Priesner (12).



Text-fig. 5. First stage larva. *T. tabaci*.

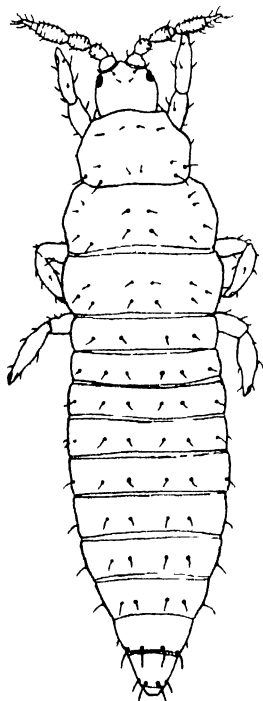
General body colour pale yellow, dorsally shaded with dark grey. Antennal segment I usually lighter than the rest, which are grey. Legs yellow, femora and tibiae usually dark grey; wings pale yellow.

*Antennae* 225–250 $\mu$ , long and slender; segment VI, 43–46 $\mu$ ; segment VII, 15–17 $\mu$ .

*Prothorax* broader than long; bristles on anterior border insignificant; bristles on posterior angles comparatively short, measuring 35–43 $\mu$ . Hind

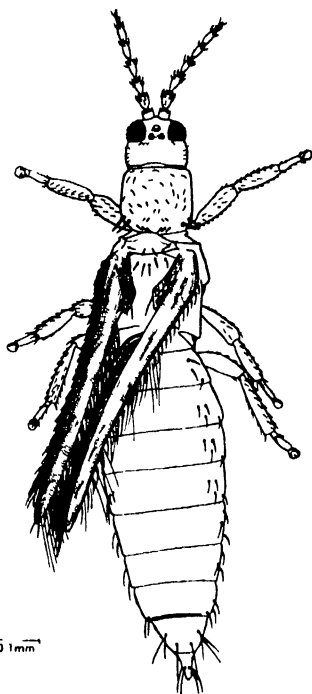


border between the angles with three small bristles on each side. *Wings* with well-developed veins; fore-wings 0.65–0.68 mm. in length. Costa with 27–30 bristles, less often with only 24–26 bristles. Main vein with 7 basal and usually 4 (2 + 2, or 2 + 1 + 1) distal bristles. Parallel vein with at most 15–17 bristles.



0.1 mm.

Text-fig. 6. Second stage larva.  
*T. tabaci*.



0.1 mm

Text-fig. 7. Adult female.  
*T. tabaci*.

*Abdomen* fairly broad; segment VIII dorsally at hind border with long complete comb of 20–29 teeth, 9–17 $\mu$ , usually 15 $\mu$  long. Bristles on segment X (dorsal) are 70–75 $\mu$  long. Ovipositor 0.17–0.18 mm. long. Body length 0.8–0.9 mm. (See Text-fig. 7.)

(d) *A delay in the development of infective power within the insect vector.*

Samuel and Bald in a recent communication (13) state that there exists an "incubation period" of 5–7 days of the spotted wilt virus within the Thrips *Frankliniella insularis*, while Linford (9), dealing with pineapple yellow spot and *Thrips tabaci*, finds an "incubation period" of about

10 days within the insect vector. The writer has also studied this question, and a series of experiments has been carried out at Cambridge to investigate a delay in the development of infective power within the Thrips, *T. tabaci*. The procedure in these experiments is similar to that used by the writer in investigating the "incubation period" of the virus of potato leaf-roll within the body of the aphid vector *Myzus persicae* (17). Batches of six and twelve non-infective larval Thrips were allowed to feed for periods of 24-72 hours upon the source of infection, usually a recently infected plant of *Datura stramonium*. The insects were then transferred in a progressive series to healthy *Datura* plants and allowed to feed for 24 hours on each plant. The Thrips were confined in the leaf cage described on p. 308, and thus kept under constant observation. Occasionally the experiments were varied and tomato, *Hyoscyamus* or tobacco plants were substituted for *Datura stramonium*. A great many experiments have been carried out upon this question of a delay in the development of infective power within the Thrips, but the results have been disappointing and they are presented here only as a progress report. So far as can be stated at present, infection first developed in the third plant in the series, which thus gives a delay of approximately 5 days, counting 48 hours on the source of infection plus 24 hours on each successive plant. There are two points which should be mentioned in connection with these experiments, the first is the very low percentage of positive infections obtained by this method as compared with ordinary spotted wilt transmissions with *T. tabaci*, and the second is the extremely mild character of the disease when it did develop. Whether this mildness is in any way connected with the rather short period of feeding upon the source of infection by the Thrips larva remains to be proved. In the case of a virus such as aster yellows, which is transmissible only by one species of insect and by no other means, an "incubation period" of the virus within the insect is perhaps to be expected. It is not, however, so easy to explain the same phenomenon in the case of spotted wilt, which is easily transmitted from plant to plant by mechanical means and is not confined to a single species of insect vector. The delay in the development of infective power in this case may possibly be of a more mechanical nature.

· (e) *Inability of the adult Thrips to become de novo a vector of the virus.*

The work of Samuel and Bald (13) and Linford (9) has shown that it is necessary for the Thrips to feed in the *larval* state upon a virus-infected host before it can become a vector of the disease, in other words the non-infective adult Thrips cannot pick up the spotted wilt virus *de novo*. A

series of experiments has been carried out to confirm this point, non-infective adults of *Thrips tabaci* being colonised upon various plant hosts affected with the spotted wilt virus and then transferred to healthy plants; in every case the plants so colonised remained healthy. This curious phenomenon is exactly the reverse of what happens in the case of aster yellows and its insect vector *Cicadula sexnotata*, and here Kunkel has shown (7) that it is the adult which can transmit the disease and not the larva, the reason given being that the incubation period of the virus in the leafhopper is longer than the larval life of the insect. It also happens occasionally that the Thrips may not transmit spotted wilt as a larva, if the insect feeds on a virus-infected plant towards the end of its larval life but retains the virus throughout pupation and then transmits as an adult (14). It seems to the writer that the inability of the adult Thrips to pick up the spotted wilt virus afresh may possibly be explained on an anatomical basis. Müller has shown that the larvae of certain Thrips (10) differ slightly in the internal anatomy from the adult. The writer has prepared a series of sections through the alimentary canals of larval and adult specimens of *Thrips tabaci* which shows that the oesophagus of the larva is considerably larger than that of the adult insect. Further work is being carried out on this point.

### III. IDENTIFICATION OF THE RINGSPOT VIRUS WITH SPOTTED WILT OF THE TOMATO.

While these studies with the ringspot virus were in progress the writer received from Mr J. Rees at Cardiff some tomato plants which were affected with what appeared to be a virus disease. A series of needle inoculations was therefore made from these plants to tobacco, *Datura* and young tomato plants, with the following results. The symptoms which developed on the tomatoes took the form of a bronzing of the young leaves followed by the development of circular reddish lesions, the whole manifestation being very similar to some of the symptoms described by Samuel, Bald and Pittman (14) for tomato spotted wilt. As regards the tobacco and *Datura* plants inoculated from the same tomato, these developed the ring and mosaic types of symptoms indistinguishable from those produced in these plants by the ringspot virus first described from *Solanum capsicastrum* (16). It appeared then from this sequence of events, firstly that the tomato plants from Cardiff were probably affected with the spotted wilt virus, and secondly that this virus and the ringspot originally found on *Solanum capsicastrum* were the same disease. In order to prove this supposition a series of cross-inoculations were carried

out by means of *Thrips tabaci*. One lot of insects was colonised upon the tomato plants from Cardiff which appeared to have spotted wilt and subsequently transferred to healthy tobacco and *Datura*. A second lot of Thrips was colonised upon tobacco and *Datura* affected with the ringspot virus from *Solanum capsicastrum* and subsequently transferred to healthy tomato seedlings. The results were as follows: the tobacco and *Datura* plants colonised with Thrips from the affected tomatoes developed a mosaic or ringspot disease in no way differing from the symptoms produced in those plants by the original ringspot virus. The tomato plants colonised with Thrips from the ringspot-affected *Datura* and tobacco developed symptoms identical with those exhibited by the spotted wilt tomatoes from Cardiff. These cross-inoculations offer fair evidence of the identification of the ringspot virus with spotted wilt of the tomato and also show that *Thrips tabaci* is a vector of this latter virus. There are not many records in the literature<sup>(10)</sup> of Thrips as vectors of plant viruses, but there do exist two well-documented cases, the first being the spotted wilt of tomato and the second "yellow spot" of pineapple which is transmitted by *Thrips tabaci* (Linford<sup>(8)</sup>). As regards the pineapple disease the possibility must be considered that it may be due to the same virus as spotted wilt, and many of the symptoms described by Linford would apply equally well to spotted wilt on certain other plant hosts. Pittman<sup>(11)</sup> in Australia was the first to suggest that *Thrips tabaci* was a vector of tomato spotted wilt, though this view was somewhat discounted in a later paper (Samuel, Bald and Pittman<sup>(14)</sup>), where the chief, if not the only, vector was considered to be another species of Thrips, *Frankliniella insularis*, although the possibility of *Thrips tabaci* also being a vector was not entirely dismissed. In the meantime the writer showed independently<sup>(18)</sup> that *Thrips tabaci* was an efficient vector of tomato spotted wilt. A later statement of Samuel and Bald<sup>(13)</sup> shows that they also have transmitted this virus in Australia by means of *T. tabaci*, thus confirming Pittman's original observation and the writer's own independent experiments.

#### IV. HOST RANGE OF THE VIRUS.

While fairly extensive studies upon the host range of spotted wilt have been carried out, the writer has not considered it worth while to attempt a systematic survey of all the susceptible plant hosts. The virus has, however, been transmitted to twenty species of the Solanaceae and to a number of other plants sufficiently diverse in character to indicate the wide host range of this disease. The plants to which the virus has

been experimentally transmitted by the writer are enumerated herewith, together with brief descriptions of the symptoms exhibited. As already outlined in the previous account of this virus (16), the symptom expression may take the form of one or more of three main types, *i.e.* concentric rings or irregular patterns (Plate XVI, figs. 1, 2), a mosaic mottling (Plate XVII, figs. 2, 3), and gross necrotic lesions of the "streak" type on the leaves and sometimes on the stem. As a rule the ring or circular necrotic lesion is the initial type of symptom, and this may be followed by the mosaic mottling and/or the necrotic lesions. The kind of symptom appears to be governed to some extent by the species of host plant and also to depend upon the degree of virulence of the virus, which in turn may be governed by the temperature and other environmental factors. There seems no doubt, also, that the virus loses in infective power with the passage of time, as it is much more difficult to transmit the disease from an old plant infected some months earlier than from a young plant recently infected. The symptoms of this disease upon a number of plant hosts have already been described (16) and they are not recapitulated here, but where necessary additional observations may be recorded. The following is a list of the various plants experimentally infected by the writer with the spotted wilt or ringspot virus.

*List of plants susceptible to the spotted wilt virus.*

Solanaceae:

*Solanum tuberosum*, potato.

*Solanum lycopersicum*, tomato.

*Solanum capsicastrum*, "winter cherry."

*Solanum melongena*, egg-plant.

*Solanum aculeatissimum*.

*Solanum nigrum*, black nightshade.

*Solanum dulcamara*, woody nightshade.

*Solanum nodiflorum*.

*Solanum laciniatum*.

*Solanum marginatum*.

*Capsicum* sp.

*Capsicum annuum*, Spanish pepper.

*Nicotiana tabacum*, tobacco, several varieties.

*Nicotiana glauca*.

*Nicotiana glutinosa*.

*Nicotiana rustica*.

*Datura stramonium*, "jimson weed."

*Solanaceae (contd.):**Hyoscyamus niger*, henbane.*Atropa belladonna*, deadly nightshade.*Petunia* sp., garden variety.*Leguminosae:*

Lupins.

*Compositae:*

Dahlias.

Asters.

Zinnias.

*Plantaginaceae:**Plantago major*, common plantain.*Solanaceae.*

*Solanum tuberosum*, potato. The symptoms of this disease upon the potato have already been described (16). In the previous paper of this series (20), however, the writer had succeeded in infecting the potato with the virus only by grafting with affected *Datura* scions. Further experiments have shown that the potato plant is easily infected by means of *Thrips tabaci*, the experimental transmissions giving almost as high a percentage of positive results as with the tomato plant. Primary symptoms take the form of pale spots on the leaves which later become ring-like and finally degenerate into irregular lesions. An additional manifestation is the development of gross necrotic lesions on the stem and sometimes on the petiole. The writer has also succeeded in infecting the potato plant by means of artificial inoculation, which was not achieved in the earlier experiments. This was accomplished by using only young, rapidly growing plants and by inoculating the youngest leaves from a young recently infected spotted wilt plant; it was found easier to infect potato seedlings by this method than plants arising from tubers. As regards the hardness of tubers sometimes associated with spotted wilt (16) this condition is still under investigation. It is of interest to find that the progeny of potato plants affected with this virus the previous season frequently give rise to healthy plants, and the writer has found the virus still viable on two occasions only after a winter spent in the resting tuber. There are two possible explanations of this, either the virus does not become completely systemic in the potato plant and thus fails to reach the tubers, or else the virus cannot easily survive the winter in the dormant potato.

*Solanum lycopersicum*, tomato. The chief economic significance of this virus lies in its relationship with the tomato. This plant is very

susceptible to the disease and is easily infected by the agency of *Thrips tabaci*; further tests with transmission by needle inoculation and by rubbing the leaves have also given a high percentage of positive results. The symptoms of spotted wilt on the tomato have been well described and figured by Samuel, Bald and Pittman<sup>(14)</sup> in Australia, so that detailed descriptions are not given here. It may be worth while, however, recording a few additional observations upon tomato made by the writer with his virus, which is considered to be the same as that occurring in Australia. Allowing for the probable effect of differences in environment the symptom expressions upon tomato of the writer's spotted wilt virus and that described by the Australian workers are very similar. The following is a brief account of the chief symptoms as observed at Cambridge. The initial signs of the disease appear as a slight intensification or thickening of the veins of the youngest leaves (Plate XIV, fig. 1), this is sometimes accompanied by one or two concentric rings; at about the same time the young leaves exhibit a tendency to curl slightly downwards and inwards (Plate XIV, fig. 2). The next symptom is the appearance of a very characteristic bronzing of the leaves which may also be in the form of bronze-coloured circular markings (Plate XIV, fig. 3), at this stage the plant is stunted in its growth as compared with normal plants. Occasionally tomato plants are killed outright by a severe necrosis resembling streak. This usually occurs when the plant has been infected as a young seedling; normally, however, the plant is not killed. At a later stage of the disease, symptoms may take the form of a fairly bold yellowish mosaic mottling on the leaves together with some leaf distortion (Plate XIV, fig. 4). As regards the effect of the virus on the fruits, these may occasionally show rings or ring-like marks and sometimes a "froth" or "lace" effect on the skin, while on the other hand the writer has frequently observed badly diseased plants to give rise to fruits which, though few in number and poor in quality, were otherwise apparently normal.

*Solanum capsicastrum*, "winter cherry." The reaction of this plant to the disease has already been fully described; it may, however, be mentioned that it is in *S. capsicastrum* that the rings reach their most perfect symmetry (Plate XVI, fig. 1). This plant also probably plays an important part in the over-wintering of the spotted wilt virus.

*Solanum melongena*, egg-plant. Symptoms develop in young egg-plants in 10 days, appearing as clear concentric rings accompanied by a mosaic mottling; later a certain amount of necrosis develops.

*Solanum aculeatissimum*. This spiny plant is extremely susceptible. First symptoms appear in 4-5 days as gross local lesions on the inoculated

leaves; these are followed by a pronounced clearing of the veins. The next development is the appearance of a characteristic and very bold mosaic of pale yellow and dark green, and in young plants this is often followed by a severe and lethal necrosis. The writer has not observed rings on this species.

*Solanum dulcamara*, woody nightshade. *S. dulcamara* is fairly susceptible to the virus and the writer has infected it with ease by means of *Thrips tabaci*. Initial symptoms develop in 10 days in the form of zoned lesions which may be definitely ring-like. These rapidly spread until a general necrosis of the leaves has set in, the symptom expression being sometimes very similar to a severe leaf-drop streak in potato (Plate XVII, fig. 1).

*Solanum nodiflorum*. The initial symptoms of the disease in *S. nodiflorum* develop in about 14 days and appear as pale spots which gradually take on a ring-like form. These may be followed by necrosis, while a well-marked mosaic of light and dark green sometimes develops.

*Solanum marginatum*. Symptoms appear first as large ring-like lesions, followed by a certain amount of necrosis. Growth is checked for a time, but later the plant grows away from the symptoms to a very great extent.

*Capsicum annuum*. The commonest type of symptom on *C. annuum* appears to be a whitish or yellowish mosaic mottle; rings or severe necrosis have not been observed on this plant.

*Nicotiana tabacum*, tobacco. There is little to add to the description already given of the disease on tobacco<sup>(16)</sup>, except that the strain of virus originally found in 1929, which produced a ringspot disease in tobacco, now appears to be more lethal to this plant. First symptoms tend to take the form of large lesions of a zoned or faintly ring-like character, and these are usually followed by the rapid collapse and death of the plant, which shows the "scorch" type of symptom previously described<sup>(16)</sup> (Plate XV, fig. 5).

*Nicotiana glutinosa*. This plant is very susceptible to spotted wilt, initial symptoms taking the form of circular zoned lesions on the inoculated leaves (Plate XV, fig. 3). These are followed by a progressive necrosis which gradually spreads over the leaves, eventually attacking the stem; the flowers also may be affected. The disease is usually fatal to this species.

*Datura stramonium*, "jimson weed." The symptoms of the disease upon *Datura* have already been fully described<sup>(16)</sup>, but a few additional facts may be added. The writer has observed that the virus will, on occasion, produce on this plant a characteristic symptom of the "bunchy-



top" or "rosette" type, in which growth of the affected plant ceases for a period and the leaves are curled over in a typical rosette. Later, growth may or may not be resumed and the symptoms revert to the ordinary ring or mosaic form. In older plants which have been infected for some time a common symptom is the "oak leaf" effect illustrated in Plate XVI, fig. 2. *Datura* is an easy plant to infect with the virus both by Thrips and needle inoculation, and is a favourite host plant of the insect.

*Hyoscyamus niger*. This plant reacts rather violently to spotted wilt and is frequently killed by it. First symptoms develop in 10–12 days and take the form of local ring-like or zoned lesions. These develop into a general necrosis of the leaves, often followed by a general yellowing and collapse of the plant.

*Atropa belladonna*, deadly nightshade. Belladonna, which is somewhat resistant to potato viruses, is very susceptible to the spotted wilt virus. The first symptoms, which develop 4–8 days after inoculation, consist of a crinkling or ruffling of the leaf surface, followed by a mosaic mottling of light and dark green together with "savoying" of the leaf. Necrotic rings also frequently develop, particularly in the neighbourhood of veins.

*Petunia* sp., garden variety. The reaction of the *Petunia* plant to the spotted wilt virus is of unusual interest. Local lesions appear on the inoculated leaf with remarkable rapidity and regularity, usually developing within 48 hours under the writer's conditions. These lesions appear first as brown spots, later developing a darker outer ring while the centre becomes yellowish; they vary in number apparently according to the number of points at which the virus makes entrance. Owing to this extreme *local* sensitivity to the spotted wilt virus, the *Petunia* plant has proved of great value in gauging with considerable accuracy the longevity *in vitro* of the virus (see p. 324). Another point of interest is the fact that leaf inoculation, whether by needle or Thrips, usually fails to produce systemic infection of *Petunia*; if the local lesions are numerous the leaf becomes totally necrotic and drops off and no further symptoms develop, while if there are only one or two lesions they finally dry out and the leaf remains attached. Sub-inoculation from recent local lesions on *Petunia* give positive infection, but the writer has so far failed to obtain results by inoculation from old lesions. A typical case of leaf inoculation of *Petunia* with spotted wilt is illustrated in Plate XVIII, fig. 1, while fig. 2 shows the same plant 6 weeks later. Here no systemic infection has taken place, and the original inoculated leaves may be seen at the base of the plant. Plate XVIII, fig. 3, illustrates systemic infection of *Petunia* induced by needle inoculation in the stem with the spotted wilt virus. Plate XV,

fig. 2, illustrates the local lesions produced in *Petunia* by infective Thrips, and in this case also no systemic infection ensued. The writer does not claim that it is impossible to produce systemic infection of spotted wilt in *Petunia* by leaf inoculation, because this has occurred, but only rarely, under the conditions of his experiments. It is hoped that the *Petunia* will prove of value in further studies with spotted wilt, and that it will be possible to apply Holmes' local lesion technique to this plant(5).

No further inoculation tests have been carried out with the following Solanaceous plants, but a description of their reaction to the virus will be found in the writer's first paper(16): *Solanum nigrum*, black nightshade; *Solanum laciniatum*; *Capsicum* sp.; *Nicotiana glauca*.

#### *Leguminosae.*

Lupins. The virus has been transmitted to young Lupins by means of *Thrips tabaci*. Primary symptoms usually take the form of circular lesions accompanied by irregular necrotic patches. Later quite typical concentric or single rings with a central spot develop on the leaves (Plate XVI, fig. 3). Return inoculation to *Datura* from these affected Lupins gave the normal spotted wilt reaction on that plant.

#### *Compositae.*

The Dahlia. Dahlias growing in the same glasshouse as tomatoes are sometimes found affected with a virus disease which expresses itself in concentric circles on the leaves (Plate XVI, fig. 4). Inoculation from such Dahlias to *Datura stramonium* and tobacco produced symptoms indistinguishable from those given by the writer's ringspot virus from *Solanum capsicastrum* and by the spotted wilt virus from tomatoes. Cross-inoculations by means of *Thrips tabaci* have also been carried out between healthy Dahlias and tomatoes affected with spotted wilt and between Dahlias showing "ringspot" and healthy tomatoes. The results were as follows: the tomatoes colonised with Thrips from the diseased Dahlias developed spotted wilt, and the healthy Dahlias colonised with Thrips from the spotted wilt tomatoes developed concentric rings. These experiments prove that the Dahlia is a host for the spotted wilt virus. As a rule the symptoms of the disease are not very severe upon the Dahlia; usually the rings disappear with continued growth and their place may be taken by a mosaic mottling. At the same time there may be considerable loss among young Dahlia plants if the Thrips is present in sufficient numbers. The virus is systemic in the Dahlia, and tubers from infected plants usually but not invariably give rise to diseased plants in the following season. The writer has received

from various parts of the country Dahlia plants of which the leaves were affected with a bold mosaic mottling, but it has not yet been ascertained whether this is a distinct virus or not.

**Aster.** The Aster is susceptible to the spotted wilt virus when transmitted by *Thrips tabaci*. The writer has infected young healthy seedlings by means of this insect, but mechanical inoculation has not been tried. Symptoms take the form of a light and dark green mosaic mottling which is most pronounced on the youngest leaves and on the flower bracts (Plate XVII, fig. 3); there is some check to normal growth and the flowers may be small and somewhat distorted.

**Zinnia.** This plant has been infected by both needle inoculation and by *Thrips tabaci*. The symptom expression has usually been in the form of a mosaic composed of flecks of different shades of green (Plate XVII, fig. 2), but fairly clear concentric rings have also developed in some cases. Faint rings and wave-like markings on the flower petals of infected Zinnias have occasionally been observed. Samuel, in a preliminary report, also mentions that Zinnias have been found in Australia naturally infected with spotted wilt.

#### *Plantaginaceae.*

The common plantain (*Plantago major*) is susceptible to spotted wilt and positive transmission experiments have been carried out by means of *Thrips tabaci*. Fairly clear concentric rings and zoned lesions sometimes accompanied by necrosis develop on the leaves (Plate XVII, fig. 4).

#### *Urticaceae.*

The writer has a certain amount of evidence that the common stinging nettle, *Urtica dioica* Linn., is susceptible to spotted wilt; nettles colonised with infective Thrips or inoculated by the needle have frequently developed a severe necrotic disease which is usually fatal to the plant. Return inoculations, however, to other susceptible plant hosts have only produced the symptoms of spotted wilt in these plants in one instance. It cannot yet, therefore, be said definitely that the nettle is susceptible without further experimentation.

The writer has so far failed to infect the following plants with spotted wilt by mechanical inoculation: Stocks, Antirrhinums, Calendula, Beans (*Phaseolus vulgaris*).

## V. ON SOME PROPERTIES OF THE VIRUS.

(a) *Filterability.*

The experiments on the filterability of the spotted wilt virus were commenced before those on the longevity *in vitro*. When these latter experiments were started, however, it was soon discovered that the virus lost its viability outside the plant host so rapidly that the filtration experiments had to be restarted and adapted to meet this rapid loss of vitality by the virus. The procedure finally adopted was as follows: a sand and paper-pulp filter was prepared (Henderson Smith(4)), together with  $L_1$  and  $L_3$  Pasteur-Chamberland filter candles, and all the apparatus necessary for filtering made sterile and ready for immediate use. A freshly infected *Datura*, tobacco or tomato plant was then triturated in a bag made of fine organdie muslin with a small quantity of distilled water, about 2 c.c. per gm. of tissue. Four healthy plants (tobacco, *Datura* or *N. glutinosa*) were inoculated with the juice thus obtained as controls, and the juice was then passed twice through the sand and pulp filter. Another series of healthy plants was inoculated from this filtrate and the remainder of the juice was then passed directly through the  $L_1$  and  $L_3$  candles, a set of plants being immediately inoculated with the filtrate from each candle. By this method the whole process from start to finish was carried out in 20 min., which is well within the period of viability of the spotted wilt virus. Table II shows the number of filtration experiments carried out in this manner and the negative results achieved. It seems clear that the virus is not filterable by the candle method and also that this non-filterability can hardly be due to loss of potency by ageing during the process of filtration, since the longevity *in vitro* experiments prove that the virus remains viable in similarly treated but unfiltered juice for periods up to 4 hours. Nor is it likely that the lack of success is due to the size of the virus particles, since all filtrates from the  $L_1$  candle were negative, and this grade of candle is of sufficient porosity to allow passage of bacteria. It seems probable that the non-filterability of spotted wilt may be due rather to adsorption of the virus by the candle than to any other cause. In this connection some as yet unpublished work on the filtration of certain potato viruses is relevant. The writer has found that the two potato mosaic viruses, X and Y(20), differ in a very high degree in their adsorptive capacity. It is possible to separate a disease complex consisting of X and Y merely by passing the juice containing the virus complex through a kieselguhr or kaolin bed in a Buchner funnel. The almost invariable

result is that the Y virus adsorbs to the kieselguhr or kaolin, giving a "pure culture" of the X virus in the filtrate. Similarly while the X virus easily passes the  $L_1$  filter candle the Y virus will not pass, and like the spotted wilt virus is apparently not filterable by candle methods<sup>1</sup>; both the spotted wilt and the Y virus however will pass the sand and pulp filter, the latter more readily than the former. A filtration experiment was performed using the juice of a White Burley tobacco plant which was infected with, and showing symptoms of, both spotted wilt and the potato virus known as X (20). The same procedure was adopted, and as will be seen from the table, symptoms of both diseases showed in the control inoculations while the X virus alone developed in the plants inoculated with the filtrates from the sand and pulp filter and the  $L_1$  candle; in this case the X virus failed to pass the  $L_3$  although it has done so in other filtration experiments of the writer. It will also be seen from Table II that the number of successful spotted wilt infections with the filtrate from the sand and pulp filter is considerably less than with the untouched juice, and there is little doubt that passage of the sand and pulp materially reduces the concentration of the virus. The writer here wishes to make grateful acknowledgment to Prof. D. Keilin, F.R.S., who placed all the facilities for this filtration work at his disposal.

Table II.

*Filtration experiments with the spotted wilt virus. 4-6 plants inoculated in each case; a plus sign indicates a positive infection.*

Exp. No.	Source of infection	Species of experimental plant	Control inoculation with untreated juice	Sand and pulp filter	$L_1$	$L_3$	$L_5$
1	Tobacco*	Tobacco*	+++	+	Nil	Nil	Nil
2	"	"	++	Nil	Nil	Nil	Nil
3	"	"	++++	+	Nil	Nil	Nil
4	"	"	+++	Nil	Nil	Nil	No test
5	"	"	++++	+++	Nil	Nil	No test
6	"	"	++++	+++	Nil	Nil	No test
7	<i>Datura stramonium</i>	"	++	Nil	Nil	Nil	No test
8	"	<i>Nicotiana glutinosa</i>	Nil	Nil	Nil	Nil	No test
9	"	"	+++	++	Nil	Nil	No test
10	"	Petunia	++++	+++	Nil	Nil	No test
11	Tobacco* infected with spotted wilt and potato virus X	Tobacco*	++++ both viruses	++++++ = X + = spotted wilt	++++ = X spotted wilt nil	Nil	Nil

\* White Burley.

Filtration experiments taking more than 2 hours to carry out have been omitted from the table. It is of interest to note that the number of local lesions developing in plants inoculated with the sand and pulp filtrate was almost invariably less than in the control inoculations with untreated juice.

<sup>1</sup> Since filtered through a collodion membrane.

Table III.

Longevity in vitro of the spotted wilt virus; a space indicates that no inoculation was performed, a plus sign indicates a positive infection, a minus sign indicates a negative inoculation. 4-6 plants inoculated in each case.

Exp. no.	Source of infection	Species of experimental plant	Control inoculations	Aging in hours									
				1	1½	2	2½	3	3½	4	4½	5	5½
1	Tobacco*	<i>Nicotiana glutinosa</i>	+	+	+	-	-	-	-	-	-	-	-
2	"	"	+	+	-	-	-	-	-	-	-	-	-
3	"	"	+	+	+	+	-	-	-	-	-	-	-
4	"	Tobacco*	+	+	+	+	-	-	+	-	-	-	-
5	"	<i>Nicotiana glutinosa</i>	+	+	-	-	-	-	-	-	-	-	-
6	"	"	+	+	+	-	-	-	-	-	-	-	-
7	"	Tobacco*	+	+	+	+	+	-	+	-	-	-	-
8	"	<i>Nicotiana glutinosa</i>	+	+	+	+	-	-	-	-	-	-	-
9	"	"	+	+	+	+	-	-	-	-	-	-	-
10	"	Tobacco*	+	+	+	+	-	-	-	-	-	-	-
11	<i>Datura stramonium</i>	"	+	+	+	-	-	-	-	-	-	-	-
	Tobacco*	<i>Hyoscyamus niger</i>	+	+	-	-	+	-	-	-	-	-	-

Series using *Petunia* plants only for inoculation; longevity in vitro measured by appearance of local lesions.

1	Tobacco*	<i>Petunia</i>	+	+	+	+	+	+	+	+	+	+	+
2	Tomato	"	+	+	+	+	+	+	+	+	+	+	+
3	"	"	+	+	+	+	+	+	+	+	+	+	+
4	Tobacco*	"	+	+	+	+	+	+	+	+	+	+	+
5	"	"	+	+	+	+	+	+	+	+	+	+	+
6	<i>Atropa belladonna</i>	"	+	+	+	+	+	+	+	+	+	+	+

\* White Burley.

(b) *Longevity in vitro of the spotted wilt virus.*

As it was not possible under the conditions of the experiments to filter the spotted wilt virus for tests of ageing *in vitro*, the sap was extracted from an infected plant in the manner already described by straining through fine organdie muslin and stored at room temperature. At first inoculations to healthy plants were made at 24-hour intervals, but as these, with the exception of the control plants, were consistently negative, inoculations were made at hourly and half-hourly intervals. Two sets of experiments were performed. In the first various susceptible plant hosts were used such as *Datura stramonium*, tobacco, *Hyoscyamus niger* and particularly *Nicotiana glutinosa*, which is more susceptible than the other plants. In the second set of experiments only *Petunia* was used, in order to make use of its extreme local sensitivity to the virus for measuring the effect of ageing. As in the first set of experiments the plants were inoculated at hourly and half-hourly intervals. At room temperature the virus ceases to give positive reactions after 4 hours. The details of the experiments are given in Table III.

VI. A COMPARISON OF THE VIRUS OF TOMATO SPOTTED WILT WITH  
A TOMATO VIRUS OF THE "STRIPE" OR "MOSAIC" TYPE  
ASSOCIATED WITH IT.

Considerable confusion exists at the present moment as to the identity of tomato virus diseases of the "mosaic," "stripe" and "streak" types. It is probable that many of these diseases consist of mixtures of viruses, although the writer is of opinion (see also Jarrett<sup>(6)</sup>) that potato mosaic viruses are not concerned in England with tomato virus complexes under glass. There can be little doubt that spotted wilt occurs commonly in tomatoes grown commercially in this country, and that it occurs in combination with the stripe or mosaic type of tomato virus is shown by the following experiments. Through the kindness of Dr Bennett, Mr Buddin, Mr Rees and others, the writer received various tomato plants showing symptom expressions thought to be due to viruses. One batch of tomato plants sent by Dr Bennett were considered by several competent authorities to be affected with stripe and were the subject of the experiments now to be described. Inoculations from these plants by differential methods of transmission were carried out. It was found that transmission by *Thrips tabaci* gave the ordinary spotted wilt reaction on young tomato and other plants, thus showing that this virus was present; needle inoculation produced in most cases in tomatoes symptoms similar

to those shown by the source of infection while inoculation from the juice of the ripe fruits produced an entirely different disease. Further experiments soon proved that two viruses were present in the original diseased tomato plants, one of which was spotted wilt while the other was a virus of the stripe or mosaic type. It appears from these experiments as if the spotted wilt virus was not present in the ripe fruits of the tomato<sup>1</sup> as inoculation from them gave only the mosaic virus. This mosaic has been studied by the writer, and some of its properties and reactions are given here for comparison with those of spotted wilt. It is not claimed that tomato "stripe" or "streak" is always a mixture of spotted wilt and a tomato mosaic, although this combination does give a symptom picture extremely suggestive of stripe. At the moment the writer tends to agree with Jarrett<sup>(6)</sup> that tomato stripe or streak may be a single virus of the tobacco mosaic type such as the one now under discussion; at the same time it is certain that owing to the extremely infectious nature of this type of tomato virus, together with the ease of spread of spotted wilt by the ever-present *Thrips tabaci*, combinations of these tomato viruses must be extremely common; a parallel state of affairs exists in the potato mosaic group<sup>(20)</sup>. Some reactions and properties of the spotted wilt and tomato mosaic viruses will now be briefly compared:

*Symptoms.* On *tobacco*, spotted wilt gives local rings or lesions followed by other rings, a mosaic or severe necrosis and occasionally necrosis of the stem; on the same plant the tomato mosaic gives numbers of local lesions, not rings; these coalesce, forming a gross necrosis which travels up the petiole until it reaches the stem, producing thereon very severe necroses in the form of "stripes." The plant very often dies, but if it survives the secondary symptoms take the form of a mild mottling of the tobacco mosaic type, but without blistering or malformation of the leaf.

On *Petunia* by leaf inoculation spotted wilt invariably gives local lesions within 2-3 days, but systemic infection seldom develops. The tomato mosaic has not given local lesions on this plant in the writer's experiments, but develops a systemic and characteristic dark and light green mosaic, which shows first on the youngest leaves. This plant has been used by the writer for separating a complex of these two viruses.

On *tomato* the chief characteristic of spotted wilt is the bronzing of the leaves (Plate XIV, fig. 3), with the tomato mosaic a faint but

<sup>1</sup> It is of interest to find in a paper just received (*Council Sci. Indust. Res. Australia Bull.* LV, 1931) that Bald and Samuel also failed to extract the virus of spotted wilt from the ripe fruits of tomatoes.



characteristic dark green mottle develops. The writer cannot yet say for certain whether the latter virus produces *by itself* "stripes" on the stem of tomato plants similar to those which it produces on tobacco.

On *potato* (var. Arran Victory) the spotted wilt produces necrotic spots which may take a ring-like form, these later developing into gross necroses which spread throughout the haulm. The tomato mosaic produces local lesions only on the inoculated leaves and no further spread of this virus occurs. The outstanding point of difference in the symptom expression of these two viruses on differential hosts is the "ringspot" appearance associated with spotted wilt.

*Insect transmission.* Spotted wilt is transmitted by Thrips but not apparently by other insects, and the mosaic virus, so far as the writer's experiments show, is not transmissible by either Thrips or the two aphides *Macrosiphum gei* and *Myzus persicae*.

*Filterability.* Spotted wilt does not pass an  $L_1$  Pasteur-Chamberland filter, but the mosaic virus passes all grades of these candles from  $L_1$ - $L_{13}$ .

*Longevity in vitro.* Spotted wilt loses infective power within 4 hours outside the living plant, but, so far as tested, the tomato mosaic remains viable under similar conditions for many weeks. The degree of infectivity of the latter virus is also very much higher than that of spotted wilt, and it is of the same infectious nature as that possessed by the tobacco mosaic viruses.

## VII. DISCUSSION.

Although the writer's preliminary accounts (16, 18) were the first records of the occurrence of tomato spotted wilt in the British Isles, it is probable that the disease has been present in this country for some years and there is little doubt but that it has been recorded many times as tomato "mosaic," "streak" and "stripe." In the writer's opinion spotted wilt is the cause of considerable loss to the tomato-growing industry in the British Isles, although this loss, as above suggested, has been attributed to other causes. In considering the economic importance of tomato spotted wilt it will be well to emphasise the following points. It has been shown in this paper that the virus readily attacks a number of other plant hosts, some of which are frequently grown in glasshouses along with tomatoes. Ample opportunity is thus afforded to the virus for overwintering in certain plants, such as the "winter cherry" (*Solanum capsicastrum*), for example, which is much grown for table decoration and in which the virus was first discovered in England in 1929 (16). Again, the

vector, *Thrips tabaci*, is an ubiquitous cosmopolitan insect which is present, moreover, in 90 per cent. of the glasshouses of this country, and it will feed and reproduce on many different plant hosts. Furthermore the Thrips is difficult to control, as the ordinary methods of nicotine spraying and fumigation appear to have little or no effect. Cyanide fumigation will probably destroy such adults and larvae as are exposed on the leaves of the plants, but it will not affect those individuals which are pupating in the soil, nor of course will it destroy the eggs which are embedded in the epidermis of the leaf. It would seem as if here was an opportunity to develop biological control methods on similar lines to those carried out by Speyer<sup>(21)</sup> on the glasshouse whitefly. There are two records of Hymenopterous parasites of species of Thrips, one *Thripoctenus brui* recorded in France as attacking *Frankliniella robusta*<sup>(23)</sup> and the other *Thripoctenus russelli* recorded by Bagnall<sup>(1)</sup> from *Thrips tabaci* in this country. The writer has not yet succeeded in finding this latter parasite.

At the present moment there is an urgent need in plant virus research for some stable and intelligible method of classification, and the first step towards this attainment will be the linking up of those virus diseases which may be identical but have been described as separate entities owing to their being found on different host plants. The writer has previously suggested<sup>(18)</sup> that the ringspot virus, described in America by Wingard<sup>(24)</sup> and others, might be the same disease as spotted wilt, but a recent paper by Henderson and Wingard<sup>(3)</sup> seems to throw doubt on this possibility. The following facts elucidated by these workers suggest that their ringspot virus is not the same disease as spotted wilt in spite of the close similarity of symptoms: the virus in expressed juice is viable for 22 months, it is filterable through a Berkfeld filter and it appears to be more highly infectious than spotted wilt. The reactions of the virus of Henderson and Wingard to a series of differential hosts also differ from those of spotted wilt on the same hosts as the following examples show: their ringspot is normally systemic in *Petunia* by leaf inoculation (cf. spotted wilt, p. 318), it is not systemic in potato while spotted wilt is, at all events in the portions of the plant above ground, and it affects plants such as *Antirrhinum* which the writer has failed to infect with spotted wilt, and the reactions of the two viruses upon tomato are also different. Henderson<sup>(2)</sup> has shown that the American ringspot is transmissible by the seed of *Petunia* only, and the writer is now testing the question of seed transmission of spotted wilt by this plant. Probably it will be found that there are several quite distinct ringspot diseases

capable of infecting the tobacco plant, as this type of symptom appears to be an alternative expression for "mosaic" diseases in general. There remains the possibility, already suggested on p. 313, that Linford's "yellow spot" of pineapple is the same virus as spotted wilt, and it is hoped that this point may be settled in the near future.

### VIII. SUMMARY.

Further studies upon a ringspot virus are described and the following points in the work are emphasised:

1. The insect *Thrips tabaci* Lind. is found to be an efficient vector of the virus.
2. The technique and methods of Thrips management are described.
3. Evidence is presented which indicates that the ringspot virus previously described from *Solanum capsicastrum* is identical with the tomato virus known in Australia as spotted wilt.
4. The host range of the virus has been studied. All species of Solanaceae tested, *i.e.* twenty in number, were found to be susceptible to the disease, and in addition the virus has been transmitted to Lupins, Dahlias, Asters, Zinnias and Plantains.
5. The spotted wilt virus was found not to pass an  $L_1$  Pasteur-Chamberland candle, and its viability was lost after 4 hours' ageing *in vitro*. An accurate method of testing the longevity *in vitro* of the virus is obtained by making use of the special reaction of the Petunia plant to the disease.
6. A comparison is given of the virus of tomato spotted wilt with a tomato virus of the stripe or mosaic type associated with it.

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## EXPLANATION OF PLATES XIV—XVIII.

### PLATE XIV.

The four figures in this plate show spotted wilt on tomato.

- Fig. 1. The first signs of spotted wilt on the tomato. Note the thickening of the veins; two faint rings can be seen on the lowest leaf.
- Fig. 2. This photograph shows the curling inwards and downwards of the young leaves, together with the bronzing characteristic of this disease on tomato.
- Fig. 3. Bronzing of the leaves due to spotted wilt, the bronzing often taking the form of the circular markings illustrated in this figure.
- Fig. 4. A pale yellow mosaic accompanied by some leaf distortion is a common symptom of spotted wilt on tomato, especially in older plants.

### PLATE XV.

- Fig. 1. Leaf of White Burley tobacco infected by means of *Thrips tabaci* with the ringspot or spotted wilt virus. Note the concentric rings developing in close proximity to the feeding marks of the insect which appear as small white dots.
- Fig. 2. Leaf of Petunia infected by means of *Thrips tabaci*. Note the local lesions developing close to the feeding marks as in the preceding figure. The virus did not become systemic in this case.
- Fig. 3. Local lesions on a leaf of *Nicotiana glutinosa* induced by needle inoculation with the virus of spotted wilt. Note the zoned character of the lesions.
- Fig. 4. Local lesion on a leaf of White Burley tobacco inoculated with spotted wilt. Here the lesion consists of concentric rings surrounded by an outer halo of necrosis.
- Fig. 5. This photograph illustrates the "scorch" type of symptom of spotted wilt on tobacco. The plant is killed by a fierce and general necrosis.
- Fig. 6. A combination of concentric rings and necrosis induced by spotted wilt upon the tobacco plant.

### PLATE XVI.

- Fig. 1. A leaf of *Solanum capsicastrum* affected with spotted wilt. In this plant the concentric rings reach their highest degree of symmetry.
- Fig. 2. "Oak-leaf" effect of the virus upon *Datura stramonium*.
- Fig. 3. Leaf of Lupin affected with spotted wilt. Note the ring-like symptoms.
- Fig. 4. Leaf of Dahlia affected with spotted wilt. Here again the symptoms take on a ring-like character.

## PLATE XVII.

- Fig. 1. Stem of *Solanum dulcamara* affected with spotted wilt. Note the gross lesions on the leaves.
- Fig. 2. Leaves of *Zinnia* affected with the disease. Note the fleck-like mosaic. This plant also shows rings at times.
- Fig. 3. Aster plant affected with spotted wilt. Note the dark green patches on the bracts.
- Fig. 4. Leaf of *Plantago major*, the common Plantain, affected with spotted wilt. Symptoms take a ring-like form on this plant.

## PLATE XVIII.

- Fig. 1. Young *Petunia* plant showing the local lesions induced by leaf inoculation with spotted wilt.
- Fig. 2. The same *Petunia* plant illustrated in Fig. 1, photographed 6 weeks later. No systemic infection has taken place, and the original local lesions can still be seen at the base of the plant.
- Fig. 3. Systemic infection of *Petunia* with spotted wilt, induced by stem inoculation with the virus.

(Received December 9th, 1931.)

## APPENDIX.

After the MS. had gone to press, the spotted wilt virus was found occurring naturally on the following plant hosts. These records have been made available by the kind co-operation of Mr W. Buddin and Mr L. Ogilvie, who sent the writer numbers of suspected plants:

Solanaceae. *Streptosolens Jamesonii* *Browallia speciosa major*.

Campanulaceae. *Trachelium* sp., *Campanula pyramidalis*.

Tropaeolaceae. Double nasturtium (also recorded from nasturtium by Bald and Samuel).

Leguminosae. The writer has also succeeded in infecting, by means of the Thrips, the broad bean, *Vicia faba*, on which the virus produces a severe disease.

There is some evidence that the two following plants are susceptible to spotted wilt but this is not yet proved:

Scrophulariaceae. *Digitalis purpurea*, Purple Foxglove.

Begoniaceae. *Begonia* sp.



1



2



3



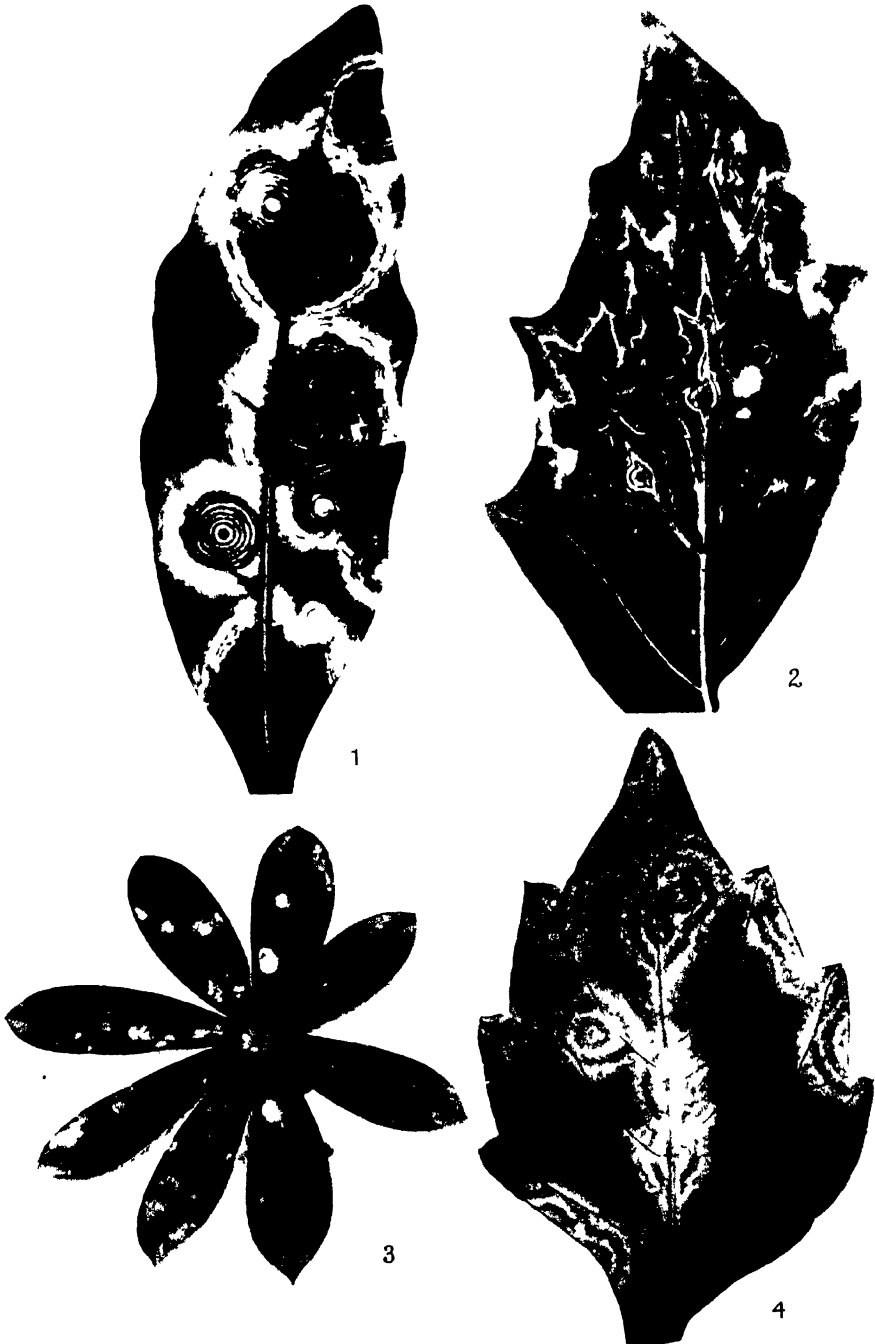
4











SMITH. —STUDIES ON PLANT VIRUS DISEASES (pp. 305-330).







## A STUDY OF *PAXILLUS PANUOIDES* FR. AND ITS EFFECTS UPON WOOD

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(With Plates XIX-XXI and 3 Text-figures.)

### OCCURRENCE.

THE fruit bodies of *Paxillus panuoides* Fr., which is synonymous with *P. acheruntius* Schroet., are not uncommon upon old sawdust heaps and upon the rotten stumps of coniferous trees, in this country and on the Continent of Europe. It is of frequent occurrence upon softwood timber in damp coal mines and has been collected from mines in South Yorkshire, Durham and South Wales, and also it is reported by Mez<sup>(8)</sup> and Liese<sup>(4)</sup> to be frequent in mines in Germany. Mangin<sup>(7)</sup> includes it in a list of the principal wood-destroying fungi of economic importance in France, and states that it occurs chiefly in mines. Occasionally it is found causing decay in buildings, and Groom<sup>(3)</sup> classifies it amongst the fungi responsible for one of the forms of dry rot; it has also been recorded as causing damage to buildings in Russia<sup>(1)</sup>. In houses, it occurs only in damp, dark situations such as cellars, and it is therefore more frequent in cold stores, ice cellars and stables, where the humidity is high. It is stated that in Sweden wooden paving is frequently decayed by this fungus.

### SPOROPHORE.

The sporophores of this fungus are fan-shaped or funnel-shaped, often imbricated and of a dingy yellow. The pileus may hang like a bell from the point of attachment or lie closely on the wood like a shell; it is at first resupinate or sessile, soon conchate, dimidiate and obovate and finally broadly expanded. The margin is at first thin and incurved, and later becomes lobed and undulating with a wavy outline. The pileus, which may measure from 2 to 10 cm. in diameter, is thin and fleshy, and the flesh is creamy becoming whitish and is soft. A stipe is not usually present, but the pileus may be extended laterally from the point where the gills radiate out excentrically. The gills are yellow and branch frequently, anastomosing behind (Plate XIX, fig. 4).

The spores are elliptical,  $4-6\mu$  by  $3\mu$ , and of an ochraceous colour. There are no cystidia.

A form of this fungus, *P. panuoides* var. *fagi*, occurring on beech has been described as a separate type, but Massee does not consider it a distinct species; it differs from the type chiefly in possessing orange gills. This variation is probably due solely to the different nature of the substratum.

#### HISTORICAL.

There is little record of experimental work upon this fungus, though it has been frequently recorded. Mez<sup>(8)</sup> states that the spores germinate readily upon slightly acid malt extract agar, and gives a brief description of the fungus in culture. Liese<sup>(4)</sup> gives a few notes regarding the fungus in culture and the microscopic appearance of the hyphae. Although it occurs in America there appears to be no record of its causing serious damage there.

#### EXPERIMENTAL WORK.

A culture of the fungus was obtained from the collection of the Forest Products Research Laboratory. This had been obtained originally from the tissue of a sporophore which appeared upon timber taken from a building near Manchester.

The sub-cultures were made upon media which had been found suitable for the growth of wood-destroying fungi. No growth took place upon malt agar (2 per cent. Keplers malt, 2 per cent. agar) and growth upon prune agar was uncertain and poor. No growth occurred on "glucose-potato-hard" medium. The fungus only made moderate growth upon the xylose medium recommended by Lutz<sup>(6)</sup> for the culture of Hymenomyces. It had been found in the case of certain wood-destroying fungi, notably *Polyporus Schweinitzii*, that considerably better growth took place when the medium was slightly acidified. Malt agar acidified with hydrochloric acid was therefore inoculated with *Paxillus panuoides*, upon which it immediately grew vigorously. The acidity of the medium was thus the factor which had been absent in the media already tried, and as this seemed a rather important factor, further work was carried out to determine the most suitable acid to be used and the optimum hydrogen-ion concentration.

*Reaction of the fungus to the acidity of the medium.*

In order to determine which acid was most favourable for the growth of the fungus varying amounts of different acids were added to 30 c.c. of sterilised 2 per cent. malt agar, and as soon as the agar had set the series was inoculated. No growth took place on media acidified with acetic or oxalic acid, and it was moderately good when tartaric or sulphuric acid was used.

The best growth took place upon the media acidified with citric or with malic acid, but it was most luxuriant upon the latter, and for most of the subsequent culture work 2 per cent. malt agar containing 1 per cent. malic acid was used. The acid was always added after sterilisation to avoid the hydrolysis and consequent liquefaction of the agar.

The favourable influence of malic acid suggested that a medium made with apples might be suitable for wood-destroying fungi. This was prepared by autoclaving 400 gm. of ripe Cox's Orange Pippins with a little water, straining off the solids and making up to 1000 c.c. with the addition of 20 gm. of powdered agar. *P. panuoides* made satisfactory growth but scarcely so vigorously as on the acidified malt. Tubes of this apple medium were inoculated with *Coniophora cerebella* and *Merulius lacrymans* and *Lenzites saepiarina*, all of them grew quite well, the two first somewhat less vigorously than on malt agar. *L. saepiarina*, however, formed abnormal fruit bodies freely and appeared to thrive on the medium.

The fungus seemed capable of withstanding a high degree of acidity, and in order to determine the range of hydrogen-ion concentration over which growth takes place a series of more accurate experiments was undertaken. Owing to the difficulty involved in the sterilisation of acidified agars, most of these were carried out upon liquid media.

*Experiments on reaction of the fungus to the acidity of the medium.*

Series of twelve conical flasks, each containing 60 c.c. of liquid nutritive extract to which varying amounts of acid had been added after sterilisation, were inoculated from a plate culture of *P. panuoides*, care being taken that the transplants of mycelium did not become submerged. A small sample was withdrawn aseptically before inoculation from each flask, and the hydrogen-ion concentration determined by means of a potentiometer, using hydrogen and calomel electrodes. The results are tabulated below.

An experiment was then carried out to determine more exactly the maximum degree of acidity at which growth can take place. Varying



amounts of *N*/1 hydrochloric acid were added to 30 c.c. of 2 per cent. malt agar in tubes, and after thorough mixing the medium was poured into Petri dishes and inoculated, a sample having been withdrawn for the determination of the *pH*. After 2 weeks' incubation at 20° C. the fungus was found to have made good growth on the plates in which the *pH* of the medium varied between 4.05 and 2.0, but no growth took place when the acidity was greater than *pH* 2.0. The maximum hydrogen-ion concentration at which the fungus can grow in 2 per cent. malt agar medium is therefore between *pH* 1.9 and 2.0.

Table I.

Exp.	Medium	Acid	No growth	Good growth
1	Potato extract	<i>N</i> /10 HCl	<i>pH</i> 7.2-4.6	<i>pH</i> 4.4-3.8
2	"	<i>N</i> /1 HCl	<i>pH</i> 7.2-4.6	<i>pH</i> 4.3-3.6
3	"	<i>N</i> /1 HCl	<i>pH</i> 6.6-4.8 1.8-1.35	<i>pH</i> 4.1-3.1
4	Turnip extract	<i>N</i> /10 H <sub>2</sub> SO <sub>4</sub>	Above <i>pH</i> 4.3	<i>pH</i> 4.3-3.9

*Description of the fungus in culture.*

Cultures of the fungus upon 2 per cent. malt agar, containing 1 per cent. malic acid, in boiling tubes were examined at frequent intervals after inoculation. Malt agar is the medium which has been adopted at the Forest Products Research Laboratory as the one on which to grow wood-destroying fungi for the purpose of standard descriptions(2); the malic acid was added to bring the medium to the *pH* most suitable for the growth of *P. panuoides*. Some of the cultures were incubated at 20° C., and others were kept in the light at laboratory temperatures.

Growth starts from the inoculum as a ball of yellowish, downy mycelium and spreads outwards slowly as a thick mat consisting of a feathery, somewhat hairy, tangled mass of very fine, forking and branching strands. The colour is at first *pinard yellow*<sup>1</sup> and later becomes *baryta yellow*. Growth is fairly slow, a boiling tube slope becoming covered in about 3 weeks at 20° C.

The mature culture shows a thick tuft of soft downy mycelium at the top, in which fruit bodies may develop, and below, very fine rather silky strands radiating from the inoculum (Plate XIX, fig. 3). In old cultures tinges of violet sometimes appear upon the mycelium and the yellow may darken to a fawn or a yellow brown. Drops of liquid which may be yellow or brown frequently appear upon the hyphae.

<sup>1</sup> Colours in italics refer to those in Ridgway's Colour Standards.

*Production of fruit bodies in culture.*

The conditions necessary for the formation of fruit bodies were not made the subjects of special study, but practically normal sporophores were freely produced in many of the cultures grown upon acidified malt agar (Plate XIX, fig. 1). A moderate sized, more or less typical fruit body was produced in a liquid medium consisting of turnip juice acidified with malic acid to pH 2.7. The first stage is the aggregation of the yellowish hyphae into a small lump which becomes closer and tighter. The smaller specimens may be cup or slipper-shaped, and the hymenial surface usually forms upon the *upper* surface; at the margin is a definite edge which may be slightly inrolled. The gills appear first as shallow folds which rapidly become deeper. The rest of the fruit body is finely pubescent. The colour of the hymenium is at first bright yellow but darkens to *ochraceous tawny* and *tawny* as the spores develop. Normal spores were produced in great numbers and in mass appeared an *ochraceous tawny* colour.

Sporophores were not produced upon cultures kept in the dark, so a certain amount of light seems to be necessary for their formation; the amount required must only be very slight because perfectly developed specimens may be found in coal mines where the only light present is that coming from miners' lamps. Neither do any exact conditions of humidity appear to be necessary for their formation.

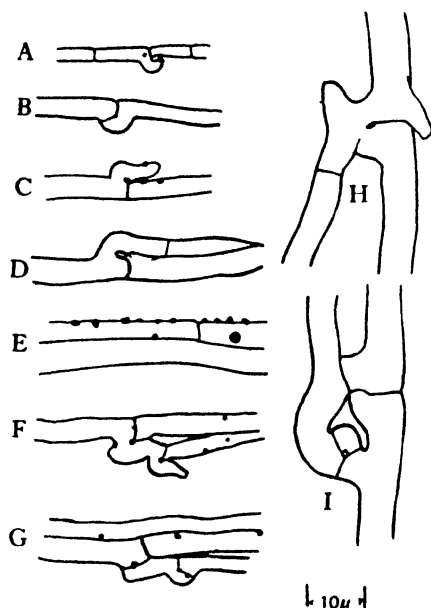
*Microscopic details of hyphae.*

The aerial hyphae are septate and vacuolate and occasionally hyaline, but usually show traces of yellow. They are usually studded with small irregular crystalline deposits, and in old cultures may secrete drops of resinous gum-like material. The hyphae are from  $2-7\mu$  in diameter measure most frequently  $4-5\mu$ . Clamp connections are frequent and there is a strong tendency to form strands by the outgrowth of branches, often from clamp connections, and by the growth of these hyphae parallel and closely appressed to the main hyphae. This process is repeated again and again until a small strand consisting of 10-20 hyphae is built up. Text-fig. 1 illustrates this development.

The mycelium submerged in the medium may be in advance of the aerial and colour the medium yellow. The submerged hyphae are more frequently branched and are hyaline or greenish yellow. Some possess yellow granular contents and oil drops, and upon the hyphae are small yellow granular lumps. They are  $3-6\mu$  in diameter, averaging  $4-5\mu$ . Clamp connections are frequent. Brownish spherical aggregations without any definite structure appear in the medium around the hyphae.

*Effect of temperature upon the rate of growth.*

In order to determine the effect of temperature upon the rate of growth and the optimum and the maximum temperatures for growth, a series of Petri dishes each containing 20 c.c. of malt agar (with 1 per cent. malic acid) of pH 2.8 was inoculated with transplants measuring approximately 4 mm. square from a young plate culture of the fungus. Eight plates were placed in each incubator at the different temperatures. The rate of growth was determined by measuring the diameter of the culture



Text-fig. 1. Hyphae of *P. panuoides* from malt agar culture showing details of clamp connections and the method of formation of strands: note the crystalline deposits on some of the hyphae.

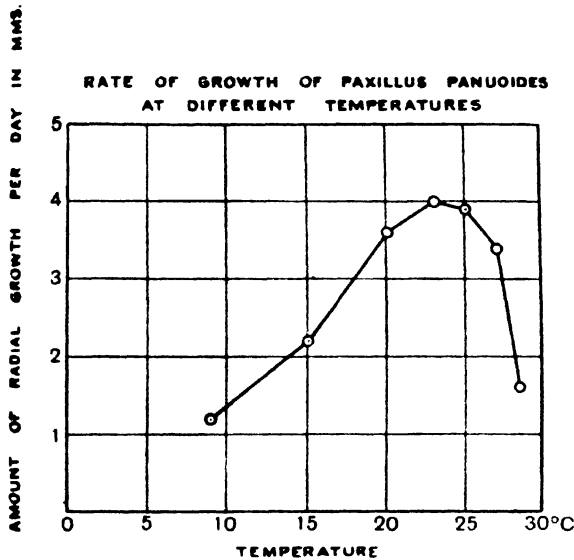
every day at noon and by subtracting the diameter found on the previous day. These increments were then averaged for each day and for the whole period and the general average was calculated.

The rate of growth in diameter of plate cultures of the fungus at different temperatures are given below:

Table II.

Temperature °C.	Rate of growth mm. per day	Temperature °C.	Rate of growth mm. per day
8-10	1.2	25	3.9
15	2.2	27	3.4
20	3.6	28.5	1.6
23	4.0	30	None

Text-fig. 2 shows a graph of the rates of growth plotted against the temperatures. It may be deduced from the curve that the optimum temperature for growth is 23–25° C. and the maximum 30° C.; it was not possible to define the lower limit for growth with exactitude but it is about 5° C. At no temperature is the rate of growth rapid as compared with that of many other fungi, and the temperature range is somewhat restricted as no growth occurs above 29° C. In possessing this restricted range, the fungus somewhat resembles *Merulius lacrymans* which, however, is capable of causing considerable damage at comparatively low



Text-fig. 2. Graph showing relation between rates of growth and temperature.

temperatures, as in cold stores just above freezing-point. Liese records that an agar culture of *P. panuoides* exposed to a temperature of 50° C. was killed after 15 min. (5).

#### *Reaction of the fungus to antiseptics.*

In order to determine the resistance of the fungus to antiseptics a number of agar tests were carried out using *P. panuoides* as the test fungus.

Varying quantities of the preservative solution were added to 20 c.c. of 2 per cent. malt extract agar containing 1 per cent. malic acid, measured from a sterilised burette, and the total volume was made up in each case to 25 c.c. by the addition of sterile water. The preservative

and the medium were well mixed in glass-stoppered bottles and poured into Petri dishes. When the medium had set it was inoculated with small transplants measuring about  $5 \times 5$  mm. The plates were incubated at  $20^{\circ}$  C. and examined at weekly intervals for 4 weeks.

The fungus was tested against ten concentrations of two well-known wood preservatives. The concentrations varied from 0.4 to 0.04 per cent. and from 0.4 to 0.008 per cent. of sodium fluoride, and from 0.6 to 0.006 per cent. (by weight) of coal-tar creosote. In no case did any growth occur on the medium which contained any preservative. From these results it is not possible to state the toxic points with exactitude, but they must lie extremely low; for sodium fluoride below 0.008 per cent., for creosote below 0.006 per cent. (compare those for *Fomes annosus* against the same antiseptics, 0.2 and 0.4 per cent. respectively) and we may conclude that *P. panuoides* is very sensitive to antiseptics and that its growth may readily be checked by a low concentration of an efficient wood preservative.

*The production of enzymes by the fungus.*

A considerable volume of mycelium was obtained from cultures upon a liquid medium, containing a 2 per cent. malt extract and 1 per cent. malic acid, upon which the fungus had made vigorous growth. The mycelial mats were thoroughly washed in distilled water, dried and then finely powdered in a mortar. A suspension of 1 gm. of this powder in 100 c.c. of distilled water was used in the following tests, and a similar suspension which had been autoclaved was used in the control tests.

*Summarised results.*

Table III.

Substrate	Test employed	Reaction	Indication
1 per cent. starch solution	Reduction of Fehling's solution	Positive, much more vigorous than in control	Presence of hydrolysing enzyme (diastase)
Alpha cellulose	Reduction of Fehling's solution	Positive and more vigorous than in controls	Hydrolysing enzyme
Hemicellulose A	Reduction of Fehling's solution	Positive and more vigorous than in controls	Hydrolysing enzyme
Glucose	Production of $\text{CO}_2$	None	Zymase absent
Amygdalin	Production of benzaldehyde	Strong	Glucoside splitting enzyme (emulsin)
Salicin	Production of reducing sugars	None in controls	
Hydrogen peroxide	Production of oxygen	Strong	Presence of catalase

No evidence of the presence of an oxidase or peroxidase in the mycelium of the fungus was obtained. The predominating enzyme seems to be hydrolytic in nature and this conclusion is strengthened by the results of the chemical analyses (see *post*). In the above table specific names have not been attached to the various enzymes hydrolysing carbohydrates since no evidence has been brought forward to show that these are in fact different substances, the hydrolysis of cellulose, cellobiose and hemicellulose, etc., may possibly be brought about by one and the same enzyme.

*Growth of the fungus upon wood.*

Several pieces of coniferous timber naturally infected with *P. panuoides* were collected, and after soaking in water were kept in glass dishes in the laboratory. The first sign of activity was the outgrowth of the soft, fibrous, yellowish mycelium in tufts, and soon fine branching strands were formed. These spread over the surface of the wood, which became covered with a soft hairy or woolly mat of yellowish colour, showing here and there tinges of violet. No thick sheets of mycelium are formed. The strands rather resemble those of *Coniophora cerebella* but are finer and remain light coloured (Plate XX, fig. 6). After the fungus had considerably decayed the blocks, normal fruit bodies were produced (see Plate XIX, fig. 4).

*Appearance of decay caused by P. panuoides.*

The first effect upon the wood produced by the fungus is a vivid yellow discoloration which appears in all parts of the wood on which mycelium is present. The colour deepens to an orange as the attack proceeds. The surface becomes softened and decay progresses from without, into the centre of the piece of wood even when mycelium is present throughout the block. In an advanced stage of decay the consistency of the wood especially in the outer layers becomes soft and cheesy. Upon drying, the decayed wood becomes light, friable and easily crumbles. It shrinks on drying and deep longitudinal fissures appear together with a number of small fine cross-cracks or checks (see Plate XIX, fig. 2). No deep cross-cracks appear as is the case in wood attacked by *Merulius lacrymans*, and the fine cross-checks are more prominent than in the decay produced by *Coniophora cerebella*.

Upon splitting open a block decayed by *P. panuoides* the outermost layers of the piece are usually seen to have decayed more than the interior, where there may be sound areas undiscoloured except for

occasional purplish pink streaks. The decay may be localised in pipes or pockets especially around the site of any crack in the original wood. The general colour of slightly decayed wood is a bright yellowish brown, but where decay has reached the final stage as on the outside of a block, the colour is a deep reddish brown.

#### *Inoculation of wood blocks.*

A number of experiments were carried out to determine the effect of *P. panuoides* upon various timbers and to provide material for the preparation of microscopic slides. Small blocks of various timbers, moistened and sterilised by autoclaving, were inoculated with pieces from agar cultures. Good growth of the fungus took place upon Sitka spruce (*Picea sitchensis*), larch and beech; poor growth upon ash. It is known that it can decompose the wood of Scots pine, but in the first experiments the samples were somewhat close grown and resinous and did not prove suitable for the fungus.

#### *Growth upon wood already partially decayed.*

It has been suggested that this fungus is often secondary in its attack and follows other fungi which have made the wood more suitable for its development. A number of experiments were carried out to determine:

(1) The amount of decomposition, measured by the loss in weight of the wood, that the fungus is capable of causing.

(2) The moisture content most suitable for growth.

(3) Whether wood already partially decayed by another fungus was more suitable than fresh wood.

(4) The effect of treating wood with dilute acid before exposing it to fungus.

*Exp. 1.* In this experiment six blocks of sound Sitka spruce wood and six blocks of the wood which had been exposed to the attack of *Trametes serialis* for 9 months were weighed and then piled alternatively in large tubes (11 × 3 in.) specially made for experiments with wood blocks. A wad of wet absorbent cotton-wool was placed at the bottom to act as a reservoir of moisture and the tubes were plugged with cotton-wool. All the blocks were inoculated from a Petri dish culture of the fungus, and were kept at laboratory temperature for 12 months. At the end of this period they were removed from the flasks, examined and weighed and, after oven-drying, reweighed.

The fungus grew vigorously upon all the blocks which were sufficiently moist and caused considerable losses in weight. The surface of the blocks

was much softened, and upon drying broke up with numerous cross-checks and deep longitudinal cracks. The greatest loss in weight, which occurred in the specimen of which the final moisture content was 87.7 per cent., was 32.9 per cent. The differences between individual blocks were so great that a general comparison between the growth upon the blocks originally sound and those already decayed was not possible. It could be definitely said, however, that previous attack by another fungus of the same type did not seem in any way to retard the attack by *P. panuoides*. In another experiment no difference was observable in the growth upon blocks of wood which had been moistened with liquid in which another fungus had been grown and on the control blocks moistened with distilled water.

Table IV.

*Loss in weight of Sitka spruce specimens exposed to  
P. panuoides for 4 months.*

Average moisture content of specimens 15.9 per cent. of dry weight when weighed before experiment.

Air-dry weight gm.	Calculated oven-dry weight gm.	Wet weight after 4 months gm.	Oven-dry weight after exp. gm.	Loss in dry weight gm.	% loss in dry weight	Average % loss in weight	Moisture content at end (%)
Controls soaked in water							
5.33	4.60	6.27	3.98	0.62	13.5	16.1	60.0
6.00	5.68	7.42	4.42	1.26	22.2		67.9
5.60	4.83	6.30	4.09	0.74	15.3		54.0
5.31	4.58	5.72	3.90	0.68	14.8		46.7
5.75	4.96	6.73	4.32	0.64	12.9		55.8
5.49	4.74	6.80	4.02	0.72	15.2		69.2
4.96	4.28	6.22	3.53	0.75	17.5		76.1
5.01	4.32	5.87	3.56	0.76	17.6		65.0
Soaked in N/50 HCl acid							
5.02	4.33	6.25	3.54	0.79	18.2	13.2	76.5
5.01	4.32	7.30	3.72	0.60	13.9		96.3
5.39	4.65	8.45	4.24	0.41	7.9		99.3
5.31	4.58	7.25	3.99	0.59	12.9		81.8
Soaked in N/20 HCl acid							
5.39	4.65	7.73	4.24	0.41	8.8	10.0	87.0
4.84	4.18	7.82	3.74	0.44	10.5		109.0
5.49	4.74	8.98	4.25	0.49	10.3		111.2
5.69	4.91	8.06	4.40	0.51	10.4		83.3
Soaked in N/10 HCl acid							
5.32	4.60	6.62	4.49	0.11	2.5	1.7	47.4
6.30	5.43	9.66	5.28	0.15	2.8		83.0
5.19	4.48	7.54	4.45	0.03	0.7		69.4
6.11	5.28	8.76	5.25	0.03	0.7		67.0
Soaked in N/5 HCl acid							
5.20	4.49	7.78	4.32	—	—	0	72.1
5.40	4.67	8.40	4.69	—	—		79.1
5.29	4.56	8.18	4.57	—	—		79.0
5.19	4.48	7.73	4.50	—	—		71.7



Table IV (contd).

Air-dry weight gm.	Calculated oven-dry weight gm.	Wet weight after 4 months gm.	Oven-dry weight after exp. gm.	Loss in dry weight gm.	% loss in dry weight	Average % loss in weight	Moisture content at end (%)
Soaked in N/2 HCl acid							
5.08	4.38	6.63	4.37	—	—	0	51.8
4.99	4.31	6.57	4.30	—	—		52.8
5.35	4.62	7.26	4.63	—	—		51.9
5.58	4.82	7.45	4.82	—	—		54.5
Soaked in N/1 HCl acid							
5.70	4.92	7.46	4.89	—	—	0	52.5
5.29	4.57	7.12	4.49	—	—		53.7
5.13	4.43	7.19	4.48	—	—		60.5
5.79	4.99	7.17	4.95	—	—		44.8
Specimens partially decayed by <i>Trametes serialis</i>							
4.88	4.48	7.01	3.97	0.59	13.2	15.8	76.6
4.10	3.76	5.13	3.00	0.76	20.2		71.0
5.13	4.70	6.30	3.83	0.87	18.5		64.5
4.42	4.05	6.05	3.61	0.44	10.9		67.6
4.72	4.33	5.67	3.42	0.91	21.0		65.8
4.14	3.80	4.91	3.13	0.67	17.7		56.9
3.96	3.63	4.90	3.17	0.46	12.7		54.5
4.22	3.87	5.30	3.30	0.57	14.7		60.6
5.15	4.72	8.11	4.33	0.39	8.3		86.9
4.45	4.08	6.29	3.14	0.94	23.0		100.1
4.22	3.87	5.27	3.29	0.58	15.0		60.1
3.98	3.65	5.45	3.07	0.58	15.8		77.5
4.70	4.30	6.25	3.57	0.73	17.0		75.0
4.33	3.97	5.96	3.45	0.52	13.1		64.0
4.14	3.80	9.41	3.45	0.35	9.2		173.0
4.78	4.39	6.36	3.48	0.91	20.7		82.9

*Exp. 2.* In view of the inconclusive results of *Exp. 1* with regard to the effect of a previous attack by another fungus on the attack by *P. panuoides*, another experiment was set up in which a much larger number of specimens was employed. In this case sticks of Sitka spruce measuring  $5 \times \frac{1}{2} \times \frac{1}{2}$  in. were used. These were inoculated by being placed directly on to an actively growing culture of the fungus in a type of Kolle culture flask which had been designed in connection with the laboratory tests of wood preservatives. It has been found that the attack of fungi upon a piece of wood is very much more certain and more vigorous when the wood is placed upon a growing culture of fungus than when it is inoculated with a small piece cut out from a culture. The specimens were weighed and sterilised as before and were exposed to fungus attack for 4 months, at the end of which period they were removed from the culture, weighed, oven-dried and reweighed and the moisture contents and loss in dry weight calculated.

In view of the favourable effect of acid upon the growth of the fungus in culture media it was decided to try the effect of an acid in the wood

upon the growth of the fungus, such acid treatment having possibly somewhat similar effects to those produced by the action of a fungus. So, in addition to the partially decomposed specimens, a number of sound specimens soaked in various concentrations of hydrochloric acid for 18 hours, were also inoculated.

The results of this experiment are given in Table IV.

### Conclusions.

The amount of decay measured by the average loss in weight caused by the fungus in the control samples was almost exactly the same as that in specimens which had already lost 25–30 per cent. of their weight by the attack of *Trametes serialis*. The removal of cellulose and the alterations brought about in the wood by this latter fungus seemed to have practically no effect upon the growth of *P. panuoides*. Unpublished work by Campbell at the Forest Products Research Laboratory has shown that this fungus brings about a typical brown rot by removal of cellulose, and generally acts upon the wood in a similar way to *Trametes serialis*. It would therefore appear that if the growth of one fungus in wood is stopped, another fungus of a similar type is able to continue the destruction of the wood in very much the same manner.

It is well known that *Merulius lacrymans* and *Coniophora cerebella* are frequently found growing together upon the same piece of wood, *Merulius lacrymans* being able to continue successfully to grow after the moisture content of the wood has been reduced to a point below the optimum for *Coniophora cerebella*. It has frequently been suggested, but upon little experimental evidence, that wood partially decayed by *C. cerebella* is more susceptible to decay by *Merulius lacrymans*, it being suggested that acids produced by *Coniophora cerebella* may stimulate the spores of *Merulius lacrymans* to germinate. There is no evidence of the production of staling products by wood-destroying fungi, in fact rather the reverse seems true—the by-products of growth such as acids, seem to render the wood more suitable for their growth—but the possibility of the destruction, during the sterilisation process, of any such substances produced by the fungus acting first, must not be overlooked.

The effect of treating the wood with hydrochloric acid was in every case to retard the growth of the fungus, the chemical appearing to act as a toxic agent and the effect of increasing the concentration was to inhibit still further the growth of the fungus till, at a concentration of acid equal to  $N/10$ , practically no growth took place. It is possible that the acidity in each case was above the optimum and that a weak organic

acid might have had a more favourable effect. It would be of interest to treat the wood with one of the acids which are produced by these fungi themselves, but there appears to be no conclusive evidence available however as to the exact nature of these acids and work on this subject is projected.

*P. panuoides* appears tolerant of a wide range of moisture contents provided the wood is definitely moist; the optimum growth takes place at a moisture content from 50 to 70 per cent. (of the oven-dry weight).

#### *Growth of the fungus upon sawdust.*

*P. panuoides* is frequently found upon old sawdust heaps in the field, and samples of sawdust from various timbers were inoculated with pure cultures of the fungus in the laboratory. The fungus made good growth upon sawdust from Scots pine and Sitka spruce, and upon sawdust from freshly felled Norway spruce (*Picea excelsa*) it made luxuriant and extremely vigorous growth.

Sawdust becomes stained a vivid orange yellow by the fungus, and as decay proceeds this colour may deepen to a dark purplish red-brown. The dust becomes friable and goes to powder under the fingers when dry.

Scots pine sawdust, which had been exposed to the fungus for a period of 3 years, was extracted with water, and the hydrogen-ion concentration of the extract determined by a potentiometer method was 2.6 and the same extract after boiling had a pH of 3.1. The fungus is therefore capable of bringing about a high degree of acidity in wood by its action thereupon.

50 gm. samples of Sitka spruce sawdust of particles of wood which passed a 60 mesh and did not pass an 80 mesh screen were inoculated in flasks with a pure culture of the fungus, and after 6 months were analysed together with samples of the sound wood by W. G. Campbell of the Forest Products Research Laboratory, Princes Risborough, who found the following results:

Original wood:							%
Cellulose	...	...	...	...	...	...	61.8
Lignin	...	...	...	...	...	...	26.5
Decayed wood:							
Loss due to decay	...	...	...	...	...	...	28.98
Cellulose (based upon original weights)	...	...	...	...	...	...	31.6
Lignin (based upon original weights)	...	...	...	...	...	...	25.0

He then examined a later stage in the decay by analysing Silver fir (*Abies alba*) sawdust (60–80 mesh), which had been exposed to decay by

*P. panuoides* for 12 months and obtained the following figures (results expressed as percentage of oven-dry weight of original wood):

Table V.

	Original wood (%)	Decayed wood (%)
Soluble in 1 per cent. alkali	12.0	27.09
Alcohol—benzene soluble	0.64	2.03
Cellulose	57.46	7.5
Lignin	29.35	24.7
Total pentosans	9.41	3.74
Pentosans in cellulose	3.18	0.27
Pentosans not in cellulose	6.23	3.47
Loss due to decay	—	56.32

It will be seen that out of the total loss of 56.3 per cent. in weight, due to fungus decay, the loss in cellulose which drops from 57.5 to 7.5 per cent. represents by far the greater part; the lignin dropping only from 29.3 to 24.7 per cent. The pentosans in the cellulose are almost entirely depleted, but more than half of the pentosans not in the cellulose remain.

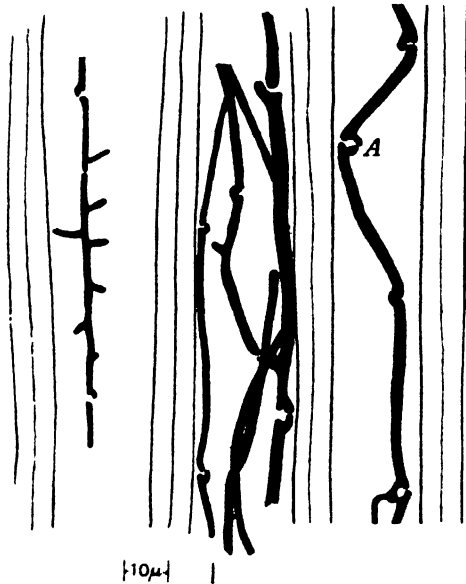
Since the carbohydrates are practically the only substances attacked, the decay may be correctly described as a "brown rot." The activity of the fungus may be closely compared with mild acid hydrolysis and there is little evidence of any oxidation. As has been shown earlier in this paper a hydrolysing enzyme is certainly present, but oxidase was not found.

*Appearance of the mycelium in wood and its effect on the cell walls.*

A number of small blocks of Sitka spruce wood were cut so that the medullary rays were parallel to the sides. These blocks were sterilised by autoclaving in tubes over water and were then laid upon actively growing cultures in culture flasks. Sample blocks were removed at different periods, varying from 5 days to 10 weeks after inoculation. They were sectioned immediately after removal from the flasks, stained and mounted.

The mycelium penetrates longitudinally into the wood along the wider tracheids and transversely along the medullary rays from which it enters the tracheids and through pits. One or more hyphae will progress rapidly along a tracheid and send out numerous side branches (Plate XXI, fig. 8). These side branches also grow along the lumen of the tracheid more or less parallel to the parent hyphae, and they continue to branch so that the whole lumen becomes blocked with a mass of hyphae running roughly parallel to the walls; in this way a sort of fine strand of mycelium is built up within the wood just as occurs in medium or on the surface of the wood. This tendency of the hyphae to be aggregated into fine strands is very characteristic of the fungus.

Frequently tracheids completely filled with mycelium may be seen next to ones which have no fungus in them (see Plate XXI, figs. 7, 8). One reason for the fungus remaining restricted to certain tracheids is that it has very little power of penetrating the cell walls. It crosses comparatively rarely from one cell to another, and when it does so it usually passes through the pits. It is quite rare to find any bore holes caused by the fungus even when decay has reached an advanced stage. This is most unusual in wood-destroying fungi, both the fungi causing "brown rots" and those causing "white rots," in nearly all the species so far investigated,



Text-fig. 3. Mycelium of *P. panuoides* in tracheids of Sitka spruce; at left the first stage, a single hypha with many side branches. Note the numerous clamp connections and the "medallion" at A.

penetrate the walls of the wood freely in all directions. Plate XXI, figs. 7 and 8, and Text-fig. 3 show the mycelium in the wood elements; the hyphae are  $1.5-4\mu$  in diameter, mostly  $2-3\mu$ , and bear numerous clamp connections. These may occasionally take the form of the so-called "medallions" in which the clamp connection is a more or less symmetrical structure (Text-fig. 3). Such "medallions" occur in the mycelium of many wood-destroying fungi, especially in those causing brown rots, and they are particularly numerous in the mycelium of *Lenzites saepiaria*, *Trametes serialis*, *Lentinus lepideus* and other fungi causing a similar type of decay.

An alteration of the substance of the cell walls by the action of the fungus, evinced by the altered reaction to stains, soon takes place, and the decayed areas take up more micro-anilin blue than safranin. This does not mean that delignification has taken place, because the phloroglucin reaction remains as vigorous as before and chemical examination (see above) has shown the reverse to be true.

#### CONCLUSIONS.

##### *Type of decay.*

The decay in coniferous timber caused by *Paxillus panuoides* may be classed as a "brown rot," as the fungus acts mainly upon the carbohydrates (cellulose) and leaves the lignin practically untouched. The wood after attack is brown and friable and splits up into flakes and small cubes.

The fungus needs a fairly high moisture content in the wood for growth (50–70 per cent. of the dry weight of the wood), but at the same time requires plenty of oxygen, and these two factors are to a certain degree mutually exclusive; if wood is saturated with moisture it cannot contain much air. It is because of these requirements that the fungus tends to attack wood superficially, for the surface layers of a piece of wood may be saturated with moisture and yet contain an adequate amount of air. This fact no doubt explains also why *P. panuoides* makes such good growth upon sawdust and is so frequently found in old sawdust heaps. Sawdust may contain a high percentage of moisture, but at the same time there is an adequate supply of air in all parts of the heap. The optimum moisture content in sawdust for the growth of most wood-destroying fungi is 100–150 per cent. (of the dry weight of the wood), far above the optimum moisture content for growth in solid wood.

##### *Recognition of decay caused by P. panuoides.*

The decay caused by this fungus may be recognised by the following features:

- (1) The tendency to decompose only the surface layers.
- (2) The discoloration produced in the wood which at first becomes stained a vivid yellow and in the final stages is dark reddish or purplish brown.
- (3) The nature of the cracking—the deep longitudinal cracks and the fine cross-checks, the latter being somewhat more pronounced than those caused by *Coniophora cerebella*.

(4) The appearance upon the wood of yellowish mycelium which is finely woolly or hairy, consisting of fine much branched strands. These strands are always fine and generally less than 1 mm. in diameter; they are at first yellow and remain light coloured, in distinction to those of *Coniophora* which become rapidly dark and blackish. The mycelium may show a bright violet tint which is very characteristic and is much more vivid than the pale lilac which sometimes appears on the silvery grey sheets of *Merulius lacrymans*.

*Prevention of decay by P. panuoides.*

Although *P. panuoides* may be classified as one of the fungi causing "dry rot" it cannot be regarded as a very serious cause of decay in buildings on account of the high moisture content required for its development. It has, however, been observed on numerous occasions upon timber removed from buildings affected with rot, and it can undoubtedly cause damage if conditions are suitable for its growth. Prevention of decay by this fungus and by *Coniophora cerebella*, which acts in very much the same way, is largely a matter of preventing the access of liquid moisture to the wood by careful attention to guttering and by remedying leaks from piping and cisterns.

Cure of an attack by this fungus should involve the removal of the decayed timber and its replacement with wood which has received some preservative treatment. Access of moisture to the timber should be stopped as far as possible, and every effort should be made to dry out the timber and the building generally by increasing the ventilation to the timber and, if necessary, by the installation of a central heating system. The control of the decay produced by *P. panuoides* does not involve the drastic measures necessary to deal with an outbreak of dry rot caused by *Merulius lacrymans*, when every trace of infection must be removed if further trouble is to be avoided. If the supply of moisture to the timber be cut off and adequate ventilation with reasonably dry air be provided, an attack by *P. panuoides* will be completely checked, and there is little danger of further outbreaks as long as the timber is kept dry.

In damp coal mines where there is much decay due to *P. panuoides*, considerable reduction of the damage may be brought about by improving the ventilation, but more sure protection is afforded by the treatment of all wood used in such mines with a preservative solution such as creosote or an aqueous solution of a salt such as zinc chloride or sodium fluoride.

## SUMMARY.

1. An account is given of the occurrence and distribution of *Paxillus panuoides* Fr.

2. The fruit bodies of the fungus produced naturally and on artificial media and the appearance of the fungus in culture are described and the microscopic details of the hyphae figured.

3. The effect of temperature, of different hydrogen-ion concentrations and of antiseptics upon the growth of the fungus are examined.

4. The growth of the fungus upon sawdust and upon blocks of sound and of already partially decayed wood are investigated and a description given of the details of the hyphae in the cells of the wood and their effect upon the walls thereof.

5. Means of recognising decay caused by *P. panuoides* and suggestions for controlling damage by this fungus are given.

## ACKNOWLEDGMENTS.

The writer is indebted to the late Prof. Groom, F.R.S., for suggesting this study, and he wishes to thank Mr K. St G. Cartwright for the kind advice given him during the course of the work. To R. S. Pearson, Esq., Director of Forest Products Research, thanks are due for permission to publish this account of the work. For assistance in the photography he is indebted to Mr Tooley and Mr Hutchins.

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## EXPLANATION OF PLATES XIX—XXI.

## PLATE XIX.

- Fig. 1. Fruit bodies of *P. panuoides* forming at edge of Petri dish culture on acid malt agar (slightly enlarged).  
Fig. 2. Typical decay produced in Sitka spruce wood by *P. panuoides* in pure culture. Note cracking, both parallel and at right angles to the grain.  
Fig. 3. Culture of *P. panuoides* upon malt agar acidified with malic acid, 1 month old. (Natural size.)  
Fig. 4. Fruit body of *P. panuoides* showing branching gills. (Natural size.)

## PLATE XX.

- Fig. 5. Petri dish culture of *P. panuoides* upon acid malt agar. (Natural size.)  
Fig. 6. Strands of *P. panuoides* upon section of joist removed from house. (About natural size.)

## PLATE XXI.

- Fig. 7. Photomicrograph of transverse section of Sitka spruce wood showing mycelium, confined to certain tracheids. (Magnified about 300 times.)  
Fig. 8. Photomicrograph of longitudinal section of Sitka spruce wood showing mycelium, confined to certain tracheids. (Magnified about 300 times.)

(Received December 17th, 1931.)



Fig. 1.



Fig. 2

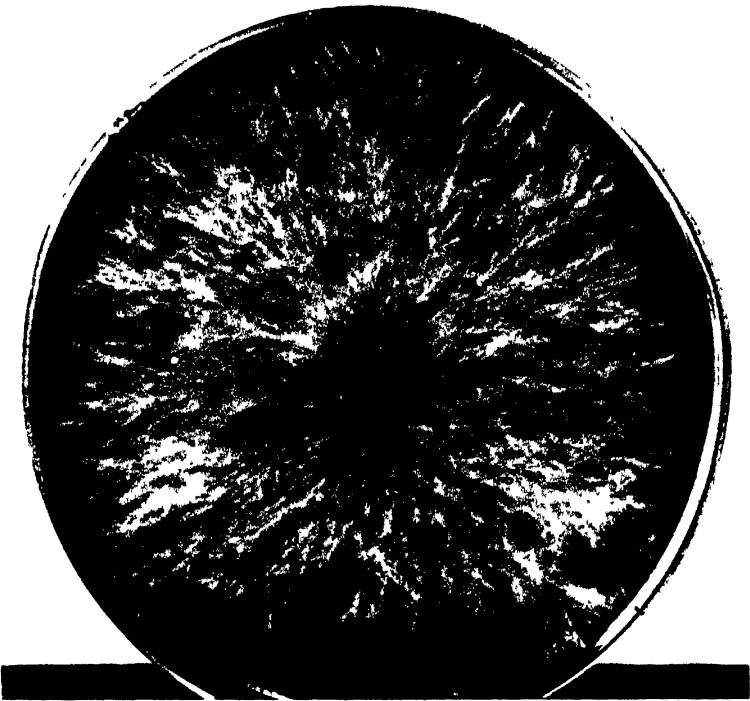


Fig. 4.



Fig. 3.





Fig

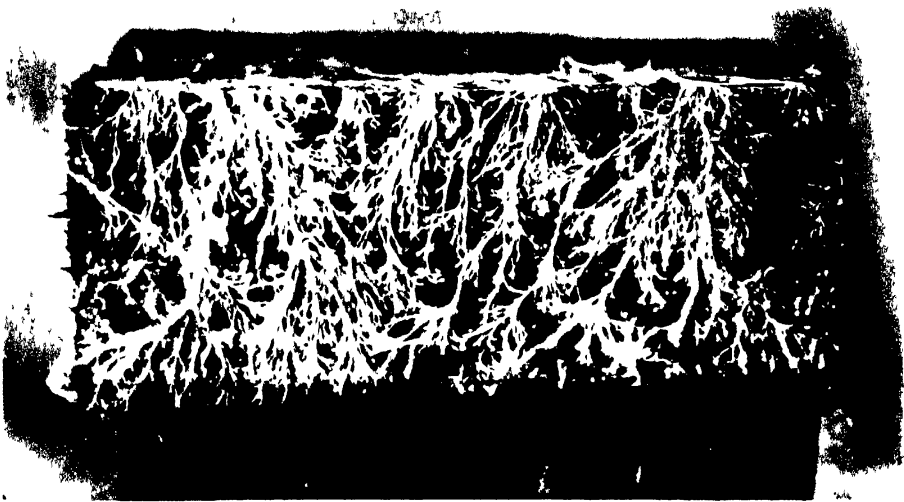


Fig. 6

FINDLAY.—A STUDY OF *P. VILLOSUS PAVONIDES* FR. AND ITS EFFECTS UPON WOOD (pp. 331-350).



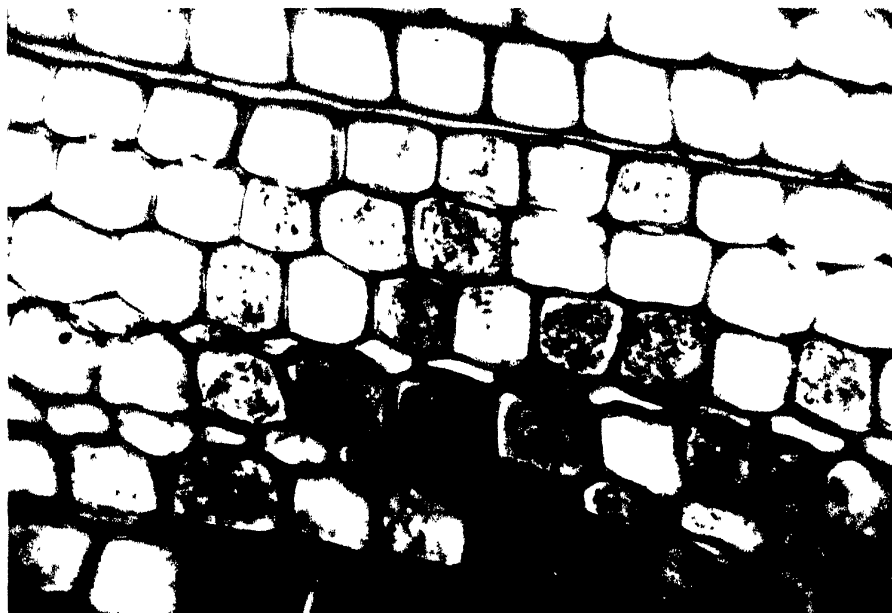


Fig. 7



Fig. 8.



# CULTURAL STUDIES ON THE *ASPERGILLI*, WITH SPECIAL REFERENCE TO LIPASE PRODUCTION OF STRAINS ISOLATED FROM STORED COPRA AND CACAO

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## I. INTRODUCTORY.

MOULDS are the cause of considerable damage to stored products and the attention paid by mycologists to plant pathology has sometimes masked the necessity for investigations on the moulds that cause deterioration of marketable produce. This damage may arise from (1) actual destruction of tissue, (2) a change in the composition of the product as in the case of the development of rancidity, or (3) the mere presence of the mould as a disfigurement.



The relative amounts of damage caused by particular strains and species of moulds occurring on any one product is usually not known. Many similar moulds play a part in the deterioration of cacao beans and copra, the limiting factor in their occurrence being the moisture content of the product. Both cacao beans and copra are high in fat content and, as it has been known for some time that fungi are capable of splitting fats by means of lipase, it was thought that an investigation of the comparative lipolytic activities of the different moulds concerned might indicate their relative importance apart from the extent to which they are found to occur.

Thirteen strains of *Aspergillus* and one each of *Mucor*, *Syncephalastrum* and *Penicillium* were therefore studied in a quantitative manner in regard to their acid and lipase-producing activities, the amount of their growth under identical cultural conditions and the influence of temperature being also considered. It was thought desirable to include a number of closely allied forms in this study, in view of the well-known fact that strains of some organisms (e.g. *Aspergillus*) which are very similar morphologically may show widely different physiological characteristics.

At the same time it was thought that the origin of the enzyme in the medium might be studied with advantage. The problem presented was to discover how far the presence of the enzyme in the medium was due to excretion and how far to the actual disintegration of the fungal cells on autolysis.

## II. HISTORICAL.

### (a) *Lipase production of fungi.*

A number of workers, using a variety of methods, have established the fact that fungi are able to hydrolyse fats, and from the first this action has been attributed to the secretion of lipase by the fungi concerned. In 1897 Gérard<sup>(11)</sup> found that *Penicillium glaucum* gave a soluble enzyme which hydrolysed monobutyrim, and in the same year Camus<sup>(3)</sup> showed that, in addition, the neutralised filtrate in which *Aspergillus niger* had been growing exerted similar powers. Deleano<sup>(8)</sup> found that *A. niger* and *A. flavus* and Garnier<sup>(10)</sup> that *A. niger*, *A. flavus* and *A. glaucus* were capable of secreting lipase. Few workers have investigated the lipolytic activities of more than one species of *Aspergillus* at the same time, and as the species selected have usually been *A. niger* and *A. flavus*

the activities of these two species have been largely regarded as typical of the group.

The only work known to the writer where large numbers of species and strains belonging to the same group of moulds were studied in a quantitative manner in regard to their lipase-producing capabilities is the unpublished work of Campbell on the *Penicillia*. Twelve species and strains were grown for from 2 to 16 weeks on three different media, and the amount of lipase present was determined at different periods of growth; the work deals with both the intra- and extracellular enzymes. Campbell showed that the lipolytic activities of one or more *Penicillia* cannot be regarded as general characteristics of the whole group.

Lipase has been found to be present in the cells of the fungus as well as in the medium in which the fungus is growing. Campbell found that the amount of lipase in the medium depends on the period of growth, and that in the case of some *Penicillia* the lipase appears in the medium after a short interval and subsequently decreases, while in the case of other strains it is only evident after a long interval. According to Garnier (*loc. cit.*) *Aspergillus fumigatus*, *A. flavus* and *A. glaucus* impart slight lipolytic activity to the medium in 2 days; this ceases at the time of fructification to appear again after 12 days. Similar observations are recorded by Went<sup>(21)</sup> for the diastase of *A. niger*. On the other hand Schenker<sup>(14)</sup> found that the lipase in the medium and dried mould of *A. niger* reached a maximum at the same time, the enzyme in the medium subsequently diminishing much more rapidly than the enzyme in the dried mould.

Schenker (*loc. cit.*) working on the lipase and Went<sup>(21)</sup> on the diastase of *A. niger*, found that the exo- and endo-enzymes reached a maximum before the dry weight. Oshima and Church<sup>(13)</sup>, on the other hand, state that the maximum protease production coincides with the maximum amount of growth. These workers made observations only for limited periods, no allowance being made for the excessive autolysis which occurs when fungi have been in culture for several weeks.

The production of particular intracellular enzymes of *Aspergillus* and *Penicillium* was found by Dox<sup>(7)</sup> to be stimulated by the presence of the specific zymolyte in the medium. Kylin<sup>(12)</sup> found the same to be the case for *Aspergillus niger* in regard to diastase, maltase and invertase. Deleano (*loc. cit.*) went still further in suggesting that the production of a specific lipase by *A. niger* and *A. flavus* was stimulated by the presence of the specific ester. Campbell found that when olive oil was substituted for sucrose as the source of carbon the production of lipase was increased.

(b) *Acid production.*

The production of acid by moulds has been studied by many workers, the fungus selected by many of them for their investigations being *Aspergillus niger*, strains of which are known to produce large quantities of oxalic acid in suitable media. The production of comparatively large quantities of oxalic acid was found by Wehmer<sup>(20)</sup> to be peculiar to *A. niger*. *A. glaucus*, *Botrytis cinerea* and *Rhizopus nigricans* produced only traces of the acid and then only when the reaction of the medium was kept near the neutral point. Schenker (*loc. cit.*) found that *Aspergillus niger* produces oxalic acid when the source of carbon is olive oil, oleic acid or glycerine.

Falck and Kingma<sup>(9)</sup> have noted that the darker coloured forms of *Aspergillus* form more acid than the lighter, and Campbell has found the same relationship to hold for the *Penicillia*. The colour of the medium was regarded by Dox<sup>(8)</sup> as an indication of the extent of autolysis, the amount of brown colour invariably being proportional to the nitrogen content in the medium. Currie<sup>(4)</sup> found that the medium in which he grew *Aspergillus niger* decreased in acidity after the tenth or eleventh day.

According to Wehmer (*loc. cit.*) low temperatures increase the production of acid by moulds.

Currie and Thom<sup>(5)</sup> studied the acidity produced in a modified Czapek medium by different strains belonging to the *A. niger* group; these strains exhibited marked differences in their capacity for acid production, the conclusion being that the strains differed in regard to rate and quantity of activity but not in regard to the type of reaction they were capable of initiating. A group characterisation was regarded as being more useful than the attempt to describe strains separately. Schiemann<sup>(15)</sup> decided that the *A. niger* as commonly understood should be classed as an unstable and mutating group.

(c) *Growth of moulds on oil.*

Biffen<sup>(1)</sup> was the first to note that the pungent odour of stale coconut oil was caused by the action of moulds, and later Walker<sup>(19)</sup> recorded the fact that the rancidity of coconut oil was principally due to the same cause. Coconut oil contains only a small proportion of unsaturated glycerides and under ordinary conditions does not easily deteriorate. Stokoe<sup>(18)</sup> made a comparison between the action of the lipase from the castor-oil bean and that from *Penicillium politans*, finding that the

former only made the coconut oil stale and "off" while the enzyme extracted from the mould produced the typical "perfume" odour and strong taste of rancid oil. He concluded that the "perfume" odour form of rancidity is brought about by an enzyme secreted by certain moulds acting probably in conjunction with lipase secreted by the same organism, and in a later paper (17) he attributed this "perfume" odour to the production of methylamyl ketone.

Brill, Parker and Yates (2) found that *Aspergillus niger* and *A. flavus* grown on freshly grated coconut meat were capable of causing a very considerable loss of oil. They regarded these two moulds as the most important in the deterioration of copra, a species of *Penicillium* being found to be unimportant. The latter was found on comparatively dry copra and its presence was a sign that the copra lacked sufficient moisture to support the growth of *Aspergillus niger* and *A. flavus*. These workers paid no attention to other moulds which, although not found to occur as often or to the same extent, might conceivably be the cause of just as much damage under other conditions.

### III. EXPERIMENTAL.

#### (a) *Fungi used and method of culture.*

The fungi used were isolated from stored products, mainly copra and cacao beans. The list below gives the series number and name of the fungi with the products from which they were isolated.

(1) <i>Aspergillus niger</i> (copra)		Isolated from stored copra	
(2)	" <i>niger</i> (cacao)	"	" cacao beans
(3)	" <i>niger</i> (tobacco)	"	" tobacco leaves
(4)	" <i>niger</i> (rubber)	"	" sheet rubber
(5)	" <i>flavus</i> (light)	"	"
(6)	" <i>flavus</i> (dark)		
(7)	" <i>tamarii</i>	"	" copra
(8)	" <i>fumigatus</i>	"	" copra
(9)	" <i>sydowi</i>	"	" cacao beans
(10)	" <i>repens</i>	"	" cacao beans
(11)	" <i>chevalieri</i>	"	" copra
(12)	" <i>terreus</i>	"	" cacao beans
(13)	" <i>ochraceus</i>	"	" sheet rubber
(14)	<i>Mucor racemosus</i>	"	" copra
(15)	<i>Syncephalastrum cinereum</i>	"	" copra
(16)	<i>Penicillium</i> species (blue-green)	"	" copra

The four strains of *Aspergillus niger* showed no distinct morphological differences. The majority of the fungi found on copra and all those found on the other products were identified by Mr R. H. Bunting as belonging to the group species under which they are quoted.

The occurrence of the moulds concerned in the deterioration of the different stored products will not be considered in this paper beyond mentioning that *A. flavus* and *A. niger* were the species most in evidence on the samples of copra examined. *A. chevalieri* and *A. fumigatus* appeared to be of less importance, while *A. tamaris* was very much in evidence on one lot of samples. The species of *Penicillium* isolated from copra was found on an otherwise fairly clean product.

The strains originally isolated from the different products were used throughout the work. The fungi were cultured and kept on Czapek agar slants, the sub-cultures being inoculated with spores from a single conidial head.

### (b) *Experimental methods.*

#### *Medium.*

The medium used was that of Czapek, as modified by Thom and Church<sup>(18)</sup> who regard it as the most suitable for the culture of *Aspergilli*. The composition is as follows:

Sucrose	...	...	...	...	...	30 gm.
Dihydrogen potassium phosphate	...	...	...	...	...	1 gm.
Magnesium sulphate	...	...	...	...	...	0.5 gm.
Potassium chloride	...	...	...	...	...	0.5 gm.
Ferrous sulphate	...	...	...	...	...	Trace
Distilled water	...	...	...	...	...	1000 c.c.

The fungi were grown in 500 c.c. hard-glass Erlenmeyer flasks, each flask containing 200 c.c. of medium. The flasks with media were sterilised in an autoclave for 15 min. at 15 lb. pressure. After sterilisation a small amount of precipitation was observed. This, however, soon disappeared when the fungus began to grow. The reaction was such that 1 c.c. of *N*/10 sodium hydroxide was required to neutralise 50 c.c. of the medium. The hydrogen-ion concentration was 6.6.

When coconut oil was substituted for sucrose as the source of carbon 7 c.c. of refined coconut oil were added to 200 c.c. of medium. The flasks were in this case sterilised by the discontinuous steaming method.

#### *Incubation and method of inoculation.*

A method of inoculation had to be employed which insured that the flasks should each receive a comparatively large amount of inoculum, as it was observed that, within limits, the amount of inoculum had a direct bearing on the rate of formation of individual mould mats. To accomplish this 1 in. test-tubes containing 50 c.c. of distilled water and fitted with cotton-wool wads through which were inserted plugged

dropping tubes, were sterilised. A large amount of spore material from a sub-culture was introduced into one of these test-tubes of sterile water, and after agitation samples of approximately 2 c.c. were removed by the dropping tube and used for inoculating each of the culture flasks.

Incubation of the flask cultures was carried out at 27° C. and for different lengths of time, five flasks being employed for each fungus for each period of growth.

Two comparison experiments were carried out in addition, using in one case the normal medium at an incubation temperature of 40° C. and in the other the coconut oil medium at 30° C. In each case three culture flasks were employed.

*Removal of cultures and drying of the mould mats.*

The cultures were removed from the incubator after 2 weeks' growth, some strains being grown for 4 and 8 weeks in addition. When coconut oil was substituted for sucrose the incubation period was 4 weeks.

Each culture was filtered through a tared No. 1 filter paper in which the fungal mat was collected and washed with distilled water. The mould mats were first dried in air at laboratory temperature and then for 24 hours *in vacuo* over calcium chloride. To compensate for evaporation the filtered medium was made up to the original volume of 200 c.c. with distilled water.

*Determination of lipolytic activity, titratable acidity and hydrogen-ion concentration of the medium.*

The lipolytic activity of the medium was determined by the hydrolytic action of the lipase on a triacetin solution and the titration of the resulting acetic acid. To 10 c.c. of medium contained in a 100 c.c. Erlenmeyer flask and neutralised with *N*/10 NaOH to a faint pink colour, were added 10 c.c. of a freshly mixed 1 per cent. triacetin solution and three drops of toluene to inhibit the growth of micro-organisms. The flask was tightly corked and maintained at a temperature of 27° C. for 3 days, after which the acid produced was estimated by titration with decinormal alkali. A similar experiment was carried out as a control, but for this purpose the lipase was destroyed by heating the medium in a steam steriliser for 30 min. The difference in the volumes of alkali required to restore neutrality in the two experiments was used to give a measure of the lipolytic activity of the medium. One determination was made for each of the five flask cultures comprising each main experiment; when only three cultures were used two determinations were made on the medium in each flask.

The volume of decinormal alkali which was required to neutralise the medium before the addition of the triacetin gave the titratable acidity.

All titrations throughout the work were made with a micro-burette using phenolphthalein as an indicator.

The hydrogen-ion concentration in the medium was determined with a Hellige Comparator, the results being accurate to 0.2 of a unit.

The lipolytic activity of each separate mould mat was not determined, but a sample was taken from a mixture of the mould mats from the flasks comprising each experiment. The fungal mats when dry were easily broken up in a mortar. A sample of 1-2 gm. was then taken and ground to a fine powder with an equal weight of fine quartz sand. To 0.1 gm. of this mixture in a small Erlenmeyer flask 10 c.c. of 0.5 per cent. triacetin solution were added. The subsequent procedure was the same as that adopted in the case of the medium, the measure of lipolytic activity being obtained in the same way. For each result five samples of the mixture of powdered sand and mould mat with an equal number of inactivated controls were employed.

(c) *Experimental results.*

(1) *The dry weight of fungus mat.*

The dry weight of mycelium produced by the different fungi over periods of weeks and at two different temperatures is illustrated in Table I. The figures are the average weight of fungal mat in the five flask cultures comprising each experiment except for the experiments at 40° C., where the figures are the averages for three flasks.

Table I.

*Weight of dried mould in grams.*

Series No. and species	2 weeks		4 weeks,	8 weeks,
	27° C.	40° C.	27° C.	27° C.
(1) <i>A. niger</i> (copra)	1.64	1.86	1.58	0.31
(2) <i>A. niger</i> (cacao)	1.63	2.00	—	—
(3) <i>A. niger</i> (rubber)	1.67	1.90	—	—
(4) <i>A. niger</i> (tobacco)	1.66	1.98	—	—
(5) <i>A. flavus</i> (light)	1.35	1.30	1.65	0.27
(6) <i>A. flavus</i> (dark)	1.00	—	—	—
(7) <i>A. tamaris</i>	1.57	1.35	1.68	1.61
(8) <i>A. fumigatus</i>	1.31	1.78	1.22	1.10
(9) <i>A. sydowi</i>	1.18	—	1.15	1.03
(10) <i>A. repens</i>	—	—	0.38	0.36
(11) <i>A. chevalieri</i>	—	—	0.80	0.62
(12) <i>A. terreus</i>	1.06	—	—	—
(13) <i>A. ochraceus</i>	1.13	—	—	—
(14) <i>M. racemosus</i>	0.54	—	—	—
(15) <i>S. cinereum</i>	1.13	—	—	—
(16) <i>Penicillium</i> sp.	0.84	—	—	—

Nearly all the fungi used showed strong growth after 2 weeks. This was especially true of the four strains of *A. niger*. *A. flavus* (dark), *A. sydowi*, *A. terreus* and *A. ochraceus* were somewhat slower in starting than the others. *A. repens* and *A. chevalieri* were exceptional in that there was no sporulation after 2 weeks, and no formation of mould mat until the third week of culture.

The seven strains cultured for longer periods showed no signs of autolysis after being in culture for 4 weeks, while after 8 weeks there was a decrease in the weight of mould mat which was, however, pronounced only in the case of *A. niger* (copra) and *A. flavus* (light). Autolysis was conspicuous in the case of these two fungi, the mould mats having become thoroughly disintegrated. The depth of colour of the medium in every case increased with the ageing of the cultures.

Five cultures of *A. niger* (copra) were grown for a period of 12 weeks, at the end of which time the average weight of mould mat was 1.60 gm. This conflicting result suggests either that the fungus was late in starting to grow or that the cultures of *A. niger* (copra) and *A. flavus* (light), weighed at 8 weeks, did not show the normal weight after 2 and 4 weeks. The work of Dox (*loc. cit.*) on the relation of autolysis to the nitrogen content in the medium would indicate that these conflicting results may be due to slight variations in the amount of nitrogen originally supplied to the medium.

Over a period of 2 weeks, the amount of growth obtained at 40° C. was somewhat higher than that at 27° C. for most of the seven strains so tested. This result indicates that the cardinal points for temperature of these moulds are definitely high.

(2) *The titratable acidity and hydrogen-ion concentration in the medium.*

The titratable acidity and hydrogen-ion concentration in the medium was tested after the fungi had been in culture for different periods of time at a temperature of 27° C. The acidity is expressed as the volume of *N/10* NaOH required to neutralise 50 c.c. of medium. The figures are an average of the acidities recorded in each of the five flasks comprising each experiment. The figure 2.8 for the hydrogen-ion concentration is to be read as 2.8 or less.

On a basis of the acidity produced in the medium after 2 weeks' growth the *Aspergilli* examined may be divided into three distinct classes:

(1) Strongly acid (25.3–38.7 c.c.): *A. niger* (copra), *A. niger* (tobacco), and *A. niger* (rubber).



(2) Medium acid (9.3–14.1 c.c.): *A. flavus* (dark), *A. flavus* (light), *A. niger* (cacao) and *A. tamarii*.

(3) Weakly acid (less than 2.0 c.c.): *A. fumigatus*, *A. sydowi*, *A. terreus*, *A. ochraceus*, *A. repens* and *A. chevalieri*.

Table II.

*The titratable acidity in cubic centimetres and the hydrogen-ion concentration in the medium.*

Series No. and species	2 weeks		4 weeks		8 weeks	
	Acidity	pH	Acidity	pH	Acidity	pH
(1) <i>A. niger</i> (copra)	25.3	2.8	47.2	2.8	1.5	7.0
(2) <i>A. niger</i> (cacao)	11.4	3.0	—	—	—	—
(3) <i>A. niger</i> (rubber)	38.7	2.8	—	—	—	—
(4) <i>A. niger</i> (tobacco)	28.3	2.8	—	—	—	—
(5) <i>A. flavus</i> (light)	10.8	6.3	28.1	2.8	1.6	7.2
(6) <i>A. flavus</i> (dark)	9.3	4.0	—	—	—	—
(7) <i>A. tamarii</i>	14.1	4.2	17.0	3.6	1.4	6.9
(8) <i>A. fumigatus</i>	0.7	7.4	1.9	6.4	2.3	6.3
(9) <i>A. sydowi</i>	1.1	7.2	0.2	7.6	0.0	8.0
(10) <i>A. repens</i>	—	—	2.0	6.0	1.6	7.0
(11) <i>A. chevalieri</i>	—	—	1.8	7.0	0.8	7.4
(12) <i>A. terreus</i>	2.0	6.0	0.7	7.6	—	—
(13) <i>A. ochraceus</i>	0.1	8.0	—	—	—	—
(14) <i>M. racemosus</i>	3.2	—	—	—	—	—
(15) <i>S. cinereum</i>	5.4	7.6	—	—	—	—
(16) <i>Penicillium</i> sp.	1.4	4.0	—	—	—	—

*A. repens* and *A. chevalieri* are included in the above classification on a basis of 4 weeks' growth.

So far as the data go, it appears that, in all cases where definitely acid conditions arise after 2 or 4 weeks' growth, there is a drift towards the alkaline side after 8 weeks. This drift is independent of the onset of autolysis of the fungal mat. Thus it is equally well shown by *A. flavus* (light) which does and *A. tamarii* which does not undergo marked autolysis after 8 weeks' growth.

On comparing the weight of mould mat after 2 weeks' growth with the acidity produced by the different fungi in the same time, it is found that those fungi producing most growth give a comparatively high acid reaction to the medium, while those fungi producing less growth alter the reaction of the medium but little.

There was a correlation between the colour of the medium, its titratable acidity and the colour of the species. The colour of the medium ranged from pale yellow when strongly acid, through amber to dark brown—in the mass port red—when nearly neutral. The species producing the most acidity and the least colour in the medium were dark-coloured ones, those producing least acidity and most colour were light-coloured,

while those imparting an amber colour were brown or green-coloured species. An exception was *A. repens* a light-coloured species, which produced little acidity and imparted a pale yellow colour to the medium. As the cultures aged there was a very noticeable increase in the depth of colour of the medium.

The influence of an increased incubation temperature on the acidity and pH of the medium produced by seven fungi after 2 weeks' growth is illustrated in Table III. The acidity is expressed as in Table II.

Table III.

*The titratable acidity and hydrogen-ion concentration in the medium at two different temperatures.*

Series No. and species	27° C.		40° C.	
	Acidity	pH	Acidity	pH
(1) <i>A. niger</i> (copra)	25.3	2.8	7.7	3.6
(2) <i>A. niger</i> (cacao)	11.4	3.0	12.8	2.8
(3) <i>A. niger</i> (rubber)	38.7	2.8	26.2	2.8
(4) <i>A. niger</i> (tobacco)	28.3	2.8	50.6	2.8
(5) <i>A. flavus</i> (light)	10.9	6.3	0.6	7.6
(6) <i>A. tamarii</i>	14.1	4.2	1.6	7.2
(7) <i>A. fumigatus</i>	0.7	7.4	2.6	7.0

On being cultured at the higher temperature *A. niger* (tobacco) and *A. fumigatus* gave increased acidity to the medium, *A. niger* (cacao) was unaffected, while the remaining fungi examined showed decreased acid-producing activities. The figures do not give any general correlation between the acidity and the amount of growth formed at the higher temperature, but it is worthy of mention that the weight of growth of *A. flavus* (light) and *A. tamarii* was little affected by the change in temperature.

When coconut oil was substituted for sucrose in the medium there was much less acid production. Under such conditions *A. niger* (copra) and *A. flavus* (light) produced negligible acidity, and *A. tamarii* gave only half the acidity shown on the sucrose medium.

### (3) *The lipolytic activity of the medium.*

The lipolytic activity of the medium on which the different fungi were cultured for different periods of time, and in the case of some species at different temperatures, is given in Table IV. The lipolytic activity is expressed as the amount of lipase found in 10 c.c. of the medium. The derivation of the figures was more fully explained under a description of experimental methods. The figures for 27 and 40° C. are the averages of five and three flask cultures respectively.

Table IV.

*The lipolytic activity in the medium.*

Series No. and species	2 weeks		4 weeks	8 weeks
	27° C.	40° C.	27° C.	27° C.
(1) <i>A. niger</i> (copra)	0.05	0.29	0.07	0.06
(2) <i>A. niger</i> (cacao)	0.03	0.09	—	—
(3) <i>A. niger</i> (rubber)	0.03	0.04	—	—
(4) <i>A. niger</i> (tobacco)	0.02	0.03	—	—
(5) <i>A. flavus</i> (light)	0.22	0.61	0.21	0.54
(6) <i>A. flavus</i> (dark)	0.04	—	—	—
(7) <i>A. tamaris</i>	0.15	0.88	0.18	0.25
(8) <i>A. fumigatus</i>	0.03	0.05	0.00	0.03
(9) <i>A. sydowi</i>	0.24	—	0.26	0.82
(10) <i>A. repens</i>	—	—	0.20	1.75
(11) <i>A. chevalieri</i>	—	—	0.15	1.17
(12) <i>A. terreus</i>	0.08	—	—	—
(13) <i>A. ochraceus</i>	0.16	—	—	—
(14) <i>M. racemosus</i>	0.05	—	—	—
(15) <i>S. cinereum</i>	0.04	—	—	—
(16) <i>Penicillium</i> sp.	0.07	—	—	—

All the fungi examined gave detectable amounts of lipolytic activity to the medium. On a basis of the lipase present in the medium after 2 weeks' growth at 27° C. the *Aspergilli* may be divided into two distinct groups: (1) those strains which impart comparatively substantial lipolytic activity to the medium, and (2) those strains which impart only sufficient activity to allow of detection by the methods employed. In the first group are *A. flavus* (light), *A. tamaris*, *A. sydowi*, and *A. ochraceus* giving from 0.15 to 0.24 unit of activity, the other fungi giving less than 0.10 unit.

The methods employed gave figures which, in the case of those fungi imparting little lipolytic activity to the medium, cannot be significantly compared, while the majority of those fungi which gave strong lipolytic activity to the medium at the same time imparted most colour and thus considerably increased the difficulties of titration and risk of inaccuracies. However, these figures are sufficiently accurate to indicate that, with at least those fungi giving strong lipolytic activity there was little change after 4 weeks, while after 8 weeks' growth there was an increased activity of medium. There is a suggestion that this increase in activity at 8 weeks may be correlated with autolysis.

No general rule was found to operate in regard to the amount of growth formed by the different fungi and the amount of lipase present in the medium.

Increasing the temperature at which the fungi were cultured was instrumental in bringing about a definite increase in the lipolytic activity

imparted to the medium by *A. flavus* (light) and *A. tamaraii*. *A. niger* (copra) shows a still more marked increase, but before this increase can be accepted as definite the experiment must be repeated as the figure is completely at variance with the general degree of lipolytic activity exhibited by the strains examined. There appears to be no connection between the relative amount of growth produced at the higher temperature and the increased activity of medium.

*A. niger* (copra), *A. flavus* (light), *A. tamaraii* and the species of *Penicillium* gave a considerably increased lipolytic activity to the medium when coconut oil was substituted for sucrose. The relative activities are not strictly comparable as the moulds were grown on the coconut oil medium at an incubation temperature of 30° C., but this increase in temperature could not altogether account for the comparatively large increase in the lipolytic activity of the medium. The coconut oil appears to have stimulated the secretion or excretion of lipase.

An experiment to determine the accuracy of the method employed was carried out on fifteen samples from the same medium with an equal number of controls. The mean activity was 0.15 with a standard deviation of  $\pm 0.05$ .

#### (4) *The lipolytic activity of the dried mould.*

The lipolytic activities of the dried mould of different fungi cultured for different periods of time, and in the case of some species at an increased temperature, are given in Table V. The figures represent the amount of lipase found in 0.1 gm. of a mixture of equal weights of sand and dried mould. The derivation of the figures was fully explained in a description of the experimental methods employed.

A test for the presence of lipase was positive in the case of all the fungi examined. The *Aspergilli* may be divided into two distinct groups on a basis of the degree of lipolytic activity in the dried mould after 2 weeks' growth (*A. repens* and *A. chevalieri* after 4 weeks): (1) Strains giving a comparatively strong reaction, 1.22–1.93 units of activity, are *A. flavus* (dark), *A. fumigatus*, *A. tamaraii*, *A. flavus* (light), *A. ochraceus*. (2) Strains giving a weak reaction, 0.40–0.57 unit of activity, are the four strains of *A. niger*, *A. sydowi* and *A. terreus*. If this classification is compared with that given on the lipolytic activity imparted to the medium, it is found that, with the exception of *A. flavus* (dark) and *A. fumigatus*, the same fungi are in the same class in each case. The two last-mentioned fungi gave but slight lipolytic activity to the medium, but showed strong activity in the dried mould.

Table V.

*The lipolytic activity of the dried mould.*

Series No. and species	2 weeks		4 weeks	8 weeks
	27° C.	40° C.	27° C.	27° C.
(1) <i>A. niger</i> (copra)	0.40	0.33	0.13	0.22
(2) <i>A. niger</i> (cacao)	0.47	0.09	—	—
(3) <i>A. niger</i> (rubber)	0.45	0.31	—	—
(4) <i>A. niger</i> (tobacco)	0.52	0.28	—	—
(5) <i>A. flavus</i> (light)	1.64	1.24	1.65	1.15
(6) <i>A. flavus</i> (dark)	1.22	—	—	—
(7) <i>A. tamaris</i>	1.53	1.14	1.56	0.94
(8) <i>A. fumigatus</i>	1.29	2.10	2.51	1.88
(9) <i>A. sydowi</i>	0.38	—	0.23	0.24
(10) <i>A. repens</i>	—	—	1.20	0.68
(11) <i>A. chevalieri</i>	—	—	1.16	0.57
(12) <i>A. terreus</i>	0.57	—	0.53	—
(13) <i>A. ochraceus</i>	1.93	—	—	—
(14) <i>M. racemosus</i>	1.05	—	—	—
(15) <i>S. cinereum</i>	1.18	—	—	—
(16) <i>Penicillium</i> sp.	0.32	—	—	—

No general rule appears to operate when the rate of growth of the different fungi is compared with the degree of activity of a sample of the dried mould. Slow-growing fungi do not necessarily secrete the least lipase. There is a suggestion that the decrease in lipolytic activity of dried mould after 8 weeks of growth is correlated with autolysis. The figures for 4 weeks' culture do not reveal any general tendency to an increased or decreased activity.

After 2 weeks' growth *A. ochraceus* was the most lipolytically active of the fungi examined.

If *A. fumigatus* is excepted the figures indicate that raising the incubation temperature to 40° C. causes a depreciation of the lipase in the dried mould. Table IV shows that at the same time there was an increase of activity in the medium. As *A. fumigatus* is a thermophilic mould it is conceivable that its secretion of lipase would be enhanced on culturing it at the higher temperature.

In an experiment which was made to determine the accuracy of the methods employed, the lipase in ten samples with an equal number of controls from the same mould mat was determined. The average activity was 1.14 with a standard deviation of  $\pm 0.11$ .

#### IV. GROWTH ON A COCONUT OIL EMULSION.

*A. niger* (copra), *A. flavus* (light), *A. tamaris* and *A. sydowi* were grown on a coconut oil emulsion, as it was thought that the relative rates

at which the different species clarified the emulsion would give some indication of their relative importance in the deterioration of copra and coconut oil.

The medium was a Czapek solution with coconut oil in emulsion as the source of carbon. At the same time the fungi were grown on a similar medium to which sucrose was added. The emulsion was made by passing coconut oil mixed with hot 95 per cent. alcohol through a hot funnel to which was attached a piece of glass tubing drawn to a fine point. The mixture of alcohol and oil was run into 250 c.c. distilled water which was kept cool and constantly stirred. Approximately 24 c.c. of oil were run into 250 c.c. distilled water but only 13 c.c. of oil went into emulsion. Before the removal of the oil floating on the surface of the emulsion the alcohol was evaporated. The 250 c.c. of emulsion were added to 250 c.c. of distilled water containing the salts necessary for the making of 500 c.c. of Czapek solution.

The moulds were grown in 10 c.c. of the Czapek solution emulsion in test-tubes which had been plugged and sterilised by the discontinuous steaming method. Three per cent. of sucrose was added to a parallel series of tubes. The tubes were inoculated with large amounts of spore material, incubated at 27° C., and examined every other day. Uninoculated controls of both media were kept for comparison. The weight of mould produced and the titratable acidity of the medium were determined. Each experiment consisted of five tubes.

From day to day the tubes were examined and the degree of opalescence compared by inspection. Before the tubes were examined they were gently shaken as some separation of the emulsion took place.

*A. flavus* (light) clarified the oil emulsion in 6 days, *A. niger* (copra) in 8 days, *A. tamaritii* in 12 days, and *A. sydowi* in 16 days. In the case of all four species the oil emulsion to which sucrose was added was clarified some days after the oil emulsion, thus indicating the protective action of sugar upon the oil. The amount of growth and acidity in the medium with sucrose added were considerably greater than on the oil emulsion. *A. tamaritii* shows a conspicuously small amount of acidity in comparison to the other three species when cultured on the oil plus sucrose medium.

## V. DISCUSSION.

The different moulds concerned in the deterioration of stored copra and cacao beans cannot all be regarded in the same light as regards their capabilities of secreting lipase. It is found that some moulds secrete relatively large amounts of lipase, often at the same time showing poor

growth, while other strains showing strong growth give relatively little lipase. For example, *A. chevalieri* and *A. repens* both produced little growth and showed strong lipolytic activity, while four strains of *A. niger* grew strongly but produced only slight activity. However, before it can be definitely stated which fungi are to be regarded as capable of causing most damage to the stored products concerned, it would be necessary to grow the different fungi on samples of the products under controlled conditions. Only a few strains were grown on a coconut oil medium, and though all were instrumental in bringing about the typical rancid odour of stale coconut oil it is clear that before the effect of an individual species can be accurately estimated, the quantity of residual oil would have to be determined. There were indications that the coconut oil had the effect of stimulating the secretion of lipase.

On the figures obtained for the amount of lipase secretion in the dried mould and the amount found in the medium, the *Aspergilli* examined divided themselves into two distinct classes, those producing a relatively strong lipolytic reaction and those producing a weak reaction. *A. fumigatus* and *A. flavus* (dark) were exceptional in that both gave but slight lipolytic activity to the medium while showing relatively strong lipase secretion in the dried mould. The methods adopted do not allow of reliable comparisons being made between fungi producing similar amounts of lipase.

The amount of growth shown by different fungi cannot be definitely correlated with the amount of lipase secreted, but there are indications that the amounts of both exo- and endo-lipases in a fungus are proportional to the amount of autolysis that has taken place. The work suggests that as autolysis sets in and the weight of mould decreases the amount of lipase found in the medium increases while that in the dried mould decreases, thus intimating that the lipase survives the disintegration of the cell tissue. Another explanation of the increased activity of the medium would be an accelerated excretion before the death of the cell. The titratable acidity of the medium appeared to be correlated with lipase production only in so far as it was an indication of the extent of autolysis. But to draw absolutely definite conclusions on the points just raised, it is considered that it would be necessary to deal with a larger number of fungi for longer periods and at the same time to make more frequent determinations; also, it would be necessary to employ methods suitable to giving figures such that small amounts of lipase could be more exactly estimated. This applies more especially to the detection of the extracellular lipase.

A further object of these experiments was to determine how far strains of the same species from different substrata exhibited different physiological activities when cultured under identical conditions. Four strains of *A. niger* showed no qualitative differences for the characteristics tested. After 2 weeks' growth at a temperature of 27° C. the strains varied considerably in regard to the acidity produced in the medium, but they all produced much the same weight of fungal mat. The four strains cannot be compared in regard to lipolytic activity because the methods employed did not give sufficiently accurate estimations of the small and similar amounts of lipase which were produced.

## VI. SUMMARY.

1. Thirteen strains of *Aspergillus*, including four of *A. niger* and two of *A. flavus*, one strain each of *Mucor racemosus* and *Syncephalastrum cinereum* and a blue-green *Penicillium* are dealt with in this paper. They were isolated from stored products, mainly copra and cacao. Determinations were made of the lipolytic activity of the dried mould, the weight of dried mould produced and the lipolytic activity, titratable acidity, and pH of the liquid Czapek medium in which the moulds were grown. The influence of an increase in temperature was also investigated.

2. Figures are given for the lipolytic activity for 2 weeks' growth and in the case of some species also for 4 and for 8 weeks' growth. All the strains were found to be lipolytically active both as regards the medium and as regards the dried mould. On a basis of 2 weeks' growth the strains of *Aspergillus* were divided into two distinct classes in relation to the amount of lipase found in the medium. In the case of the activity of the dried mould two classes were again found. With two exceptions the same moulds were found to be in the same class in both cases.

3. It was found that while the lipolytic activity of the dried mould and of the medium were materially the same at 2 weeks as at 4 weeks, determinations made at 8 weeks indicated an increase in the activity of the medium and a decrease in the activity of the dried mould coincident with a decrease in the weight of growth. It is therefore suggested that the increase in the activity of the medium is due to autolysis and probably also to an increased excretion of enzyme.

4. As it was found that the amount of lipase in a medium containing coconut oil as the source of carbon was much greater than in a sucrose medium it is concluded that the coconut oil stimulated the production of the enzyme.



5. In the case of the *Aspergilli* there was a definite correlation between the colour of the mould, the colour of the medium and the titratable acidity of the medium. The lighter coloured forms produced least acid and most colour in the medium, while the dark forms produced most acid and least colour. On autolysis the depth of colour of the medium increased and the titratable acidity decreased.

6. Marked differences in the titratable acidity of the medium were found between the four strains of *A. niger*. An increase of temperature had the effect of bringing about still more marked differences and in addition, differences in lipolytic activity. No qualitative differences were found.

7. *A. niger* (copra), *A. flavus* (light), *A. tamaris* and *A. sydowi* were grown on a coconut oil emulsion. The rates at which the different species clarified the emulsion were noted. *A. niger* and *A. flavus* clarified the emulsion in the shortest time. The addition of sucrose had the effect of considerably retarding the assimilation of the oil in the emulsion.

The writer wishes to express his thanks to Dr L. E. Campbell for giving him access to his manuscript on his work on the lipolytic activity of the *Penicillia*, to Mr R. H. Bunting, Mycologist of the Stored Products Research Laboratories, Slough, for his helpful advice and for the isolation and identification of the majority of the species used and to Prof. W. Brown for superintending the work.

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(Received December 2nd, 1931.)

# THE INFLUENCE OF ENVIRONMENTAL CONDITIONS ON THE DEVELOPMENT OF THE ANGULAR LEAF-SPOT DISEASE OF COTTON

## IV. THE INFLUENCE OF ATMOSPHERIC HUMIDITY ON INFECTION

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(With 2 Text-figures.)

EARLIER papers in this series dealt with the influence of soil temperature (2) and of air temperature (5) on the infection of young cotton plants by *Bacterium malvacearum*, the causal organism of the "Angular Leaf-Spot" or "Black-Arm" disease. The experiments were carried out in the Rothamsted control chambers, details of which have been given (1). In these chambers it is possible to control soil temperature, air temperature, air humidity, and illumination, automatically and independently over a wide range. Cotton seedlings make good growth in the chambers, and infection occurs readily under suitable conditions. In the experiments so far described the humidity has in all cases been high, always exceeding 85 per cent. and in some experiments reaching saturation. Work has now been carried out on the influence of different degrees of controlled humidity on the infection of young plants.

The seed used throughout the experiments has been "Sakellarides" variety from the Gezira Plain, supplied by the courtesy of Mr R. E. Massey, Botanist to the Sudan Government.

### DESCRIPTION OF EXPERIMENTS.

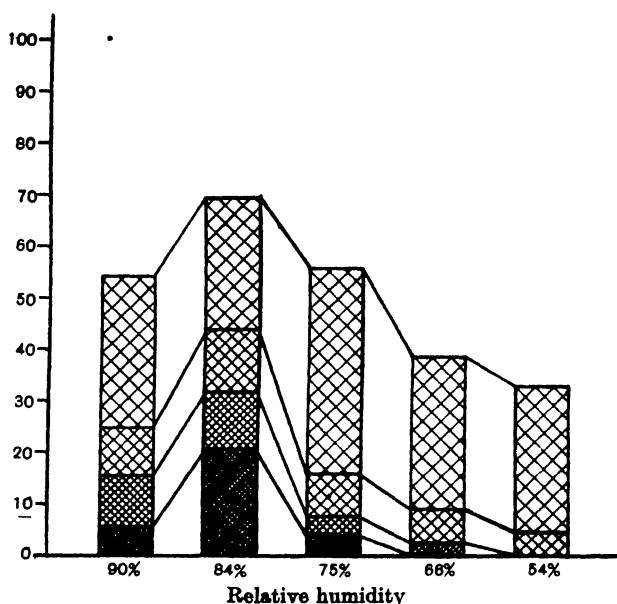
*Exp. 1.* In this experiment, owing to temporary breakdown of one of the control chambers, five of the chambers only were used. The forty soil tins were filled with Gezira cotton soil and sown, in the glasshouse, with Sakel seed, five seeds in each tin. Before sowing the seed was delinted in concentrated sulphuric acid for 10 min., washed and dried, in order to sterilise the outside of the seed. The plants were grown in the glasshouse for 6½ weeks, by which time two true leaves were fully

developed, with two or three others unfolding. The five chambers were run for several days before the plants were placed in them in order to obtain settled conditions. The previous experiments had shown that the optimum temperature conditions for infection were about 25–27° C. for the soil temperature and 30–35° C. for the air temperature. The thermostats were therefore set for 25° C. soil temperature and 30° C. air temperature. Good temperature control was obtained in all the chambers throughout the experiments, the range being usually well within 2° C. The humidity controls were set to give average relative humidities of 90, 85, 75, 65 and 55 per cent. A new type of humidifier was in use<sup>(4)</sup> and the control was quite satisfactory, with a range, in most cases not exceeding 5–6 per cent. Owing to sticking of contacts and relays temporary derangements of the controls occurred from time to time, but these were of short duration, and there is no reason to suppose that they could have any appreciable effect on the final result. The lowest humidity proved the most difficult to maintain, and varied at times between 50 and 60 per cent. The average humidity was, however, close to 55 per cent. The actual average humidities were 90, 84, 75, 66 and 54 per cent.

The plants were placed in the chambers for 3 days before inoculation to become acclimatised to the new conditions. They were then sprayed with a strong suspension of *B. malvacearum* in sterile water and left for 1 week, illumination being provided for 16 hours out of the 24. At the end of the first week no sign of infection had developed and it seemed clear that the culture used had lost its virulence. This appears to be a not uncommon phenomenon, and is possibly connected with the dissociation of the organism into strains of varying degrees of virulence, a problem which is engaging attention<sup>(3)</sup>. The plants were accordingly re-sprayed with a new young culture of the organism and left for a further period. Good infection resulted and appeared to be complete in 13 days. At the end of this time the plants were removed from the chambers and the degree of infection estimated.

The difficulty of obtaining a reasonably accurate quantitative estimate of the incidence and degree of infection was considered in the last paper of the series<sup>(5)</sup>, where the method finally adopted was given in detail. Each leaf was examined and the number of disease spots counted, due allowance being made for the cases where spots had coalesced to form a patch, or where an extended lesion occurred along a vein. The results so obtained were then grouped into four arbitrarily delimited classes: Class I, severe infection, fifty spots or more per leaf; Class II, moderate infection, twenty-five spots or more; Class III, light infection, ten spots

or more; Class IV, very light infection, less than ten spots. The results of the experiment are given in full in Table I. Where more than about seventy-five spots or the equivalent in patches occurred on a single leaf the infection was recorded as indefinite ( $\infty$ ). Text-fig. 1 shows diagrammatically the distribution of infection in the four classes at the different humidities. It is apparent that the maximum infection occurred at 84 per cent. relative humidity, but it is doubtful whether this is significantly different from the infection at 90 and 75 per cent. humidity. At humidities below these, however, there is a very marked fall in the in-



Text-fig. 1. Exp. 1. Percentage infection in four classes at various relative humidities.

cidence and severity of infection, the amount of disease becoming very small at a relative humidity of 54 per cent. From Table I it is clear that in accordance with the general rule for this disease the leaves which were unfolding at the time of inoculation (leaves Nos. 4 and 5) are usually the most heavily infected.

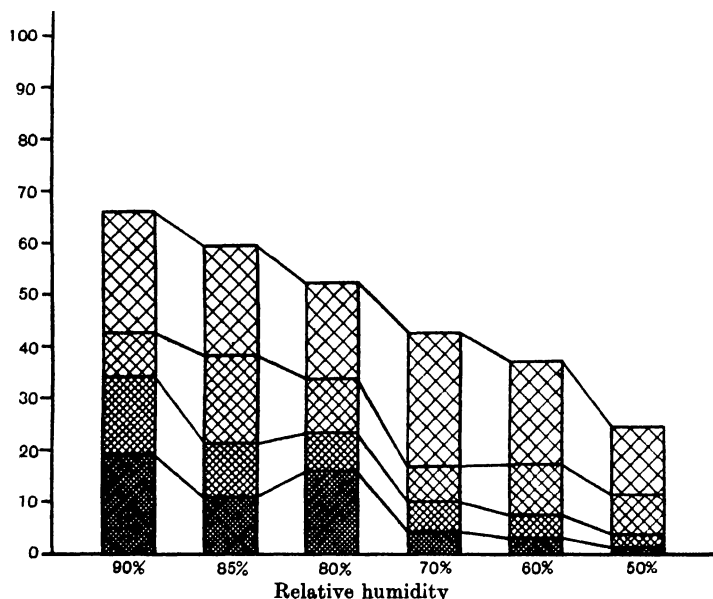
*Exp. 2.* This experiment was essentially a repetition of Exp. 1. In order to make the control of humidity easier, especially at the extremes of the range covered, one or two tins in each tank were not used for plants, but were filled, in the case of the higher humidities, with water, or for the lower humidities, with calcium chloride. As in the previous

**Table I.**  
*Exp. 1. Distribution of infection at various humidities.*

Plant no.	90 % Lead no.						84 % Lead no.						75 % Lead no.						66 % Lead no.						54 % Lead no.							
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6		
1	—	—	—	1	17	36	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
2	—	—	—	1	11	11	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
3	—	—	—	0	0	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
4	—	—	—	0	0	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
5	—	—	—	2	32	31	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
6	—	—	—	1	26	37	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
7	—	—	—	0	0	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
8	—	—	—	0	0	75	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
9	—	—	—	6	∞	45	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
10	—	—	—	20	1	37	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
11	—	—	—	11	1	45	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
12	—	—	—	0	8	0	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
13	—	—	—	0	50	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
14	—	—	—	0	8	35	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
15	—	—	—	1	20	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
16	—	—	—	0	12	75	25	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
17	—	—	—	0	0	4	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
18	—	—	—	0	2	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
19	—	—	—	0	2	25	65	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
20	—	—	—	1	1	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
21	—	—	—	3	15	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
22	—	—	—	3	2	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
23	—	—	—	3	2	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
24	—	—	—	1	5	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
25	—	—	—	1	5	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
26	—	—	—	8	—	60	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
27	—	—	—	8	2	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
28	—	—	—	4	26	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
29	—	—	—	4	18	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
30	—	—	—	5	—	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
31	—	—	—	0	0	0	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
32	—	—	—	—	22	65	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
33	—	—	—	0	16	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
34	—	—	—	0	0	0	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
35	—	—	—	0	0	0	6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
36	—	—	—	0	26	∞	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
37	—	—	—	1	1	3	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
38	—	—	—	0	5	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
39	—	—	—	1	8	25	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
40	—	—	—	2	6	70	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
Total no. of leaves			142						124						135						125						135					
Class I																																
50 spots and over																																
Class II																																
25 spots and over																																
Class III																																
10 spots and over																																
Class IV																																
Less than 10 spots																																
Total no. of leaves																																
infected																																

experiment the tins were filled with Gezira soil and sown, in the glass-house, with Sakel seed, five seeds per tin. The plants were allowed to grow in the glasshouse for 8 weeks, in which time four or five leaves had developed while two or three were still unfolding.

Six chambers were used and the thermostats in all were set for a soil temperature of 29–30° C. and an air temperature of 32–33° C. The average relative humidities in the six chambers were 90, 85, 80, 70, 60 and 50 per cent. The plants were placed in the chambers and left for 3 days to become acclimatised. The period of illumination was as in the



Text-fig. 2. Exp. 2. Percentage infection in four classes at various relative humidities.

previous experiment. On the third day all the plants were sprayed with a suspension in sterile water of a recently isolated culture of *B. malvacearum*, and left for the infection to develop. Symptoms appeared on the fifth day and the infection appeared to be fully developed in eleven days. The plants were removed from the chambers and examined leaf by leaf and the degree of infection estimated by the same method as in the other experiments. The full results are given in Table II, and the distribution of infection in the four classes is shown diagrammatically in Text-fig. 2. It will be seen that the results confirm those of Exp. 1, the decrease in infection with humidity being very regular. The intensity of attack, as distinct from the total percentage number of leaves diseased, is somewhat

*Exp. 2. Distribution of infection at various humidities.*

Plant no.	90 %					85 %					80 %					70 %					60 %					50 %									
	Leaf no.					Leaf no.					Leaf no.					Leaf no.					Leaf no.					Leaf no.									
1	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8			
" 2	—	0	7	17	∞	11	—	—	0	1	0	18	23	0	0	—	—	4	1	1	9	20	0	—	—	0	0	1	0	14	∞	—			
" 3	—	0	43	49	∞	33	3	—	0	0	0	9	54	1	0	—	—	0	0	1	0	0	—	—	—	0	0	0	0	0	0	45	0		
" 4	—	0	4	∞	∞	45	5	—	0	0	0	30	46	10	13	—	—	0	0	0	0	60	—	—	—	0	0	0	0	11	20	25	0		
" 5	0	0	0	2	4	∞	15	—	0	2	7	∞	∞	—	—	—	—	0	0	0	—	—	—	—	0	0	0	0	2	8	58	11			
" 6	0	0	0	3	4	50	—	—	0	0	0	28	12	1	0	—	—	0	0	0	—	—	—	—	0	0	0	0	2	24	∞	0			
" 7	0	0	0	0	9	∞	17	—	—	0	7	22	12	—	—	—	—	0	0	0	4	∞	—	—	0	0	0	0	2	0	18	35	12		
" 8	0	0	0	15	∞	—	—	—	0	3	18	3	—	—	—	—	—	0	0	0	4	∞	—	—	0	0	0	0	0	0	20	0	0		
" 9	—	0	39	∞	11	—	—	—	0	0	0	3	∞	1	—	—	—	0	0	0	5	9	35	—	—	0	0	0	0	0	0	20	0	—	
" 10	—	1	2	∞	30	—	—	—	0	13	12	16	44	1	—	—	—	1	3	1	∞	25	—	—	—	—	0	0	0	0	1	20	—	—	
" 11	0	0	0	0	21	∞	—	—	0	0	12	7	∞	—	—	—	—	0	0	0	—	—	—	—	—	—	0	0	0	0	0	6	1	—	
" 12	—	0	12	38	48	6	—	—	—	0	6	1	2	11	0	—	—	—	0	0	1	∞	35	15	5	—	0	0	0	0	0	0	7	5	—
" 13	—	0	6	3	40	5	31	—	—	0	0	1	5	∞	35	—	—	—	0	0	0	20	—	—	—	—	0	0	0	0	0	0	0	—	—
" 14	0	0	0	0	1	5	∞	—	—	0	0	16	∞	48	10	—	—	—	0	0	0	0	—	—	—	—	0	0	0	0	0	0	0	—	—
" 15	—	0	0	0	3	∞	35	—	—	2	3	6	∞	—	—	—	—	—	0	0	0	32	—	—	—	—	0	0	0	0	0	0	0	—	—
" 16	—	0	0	11	—	—	—	—	—	0	0	0	26	0	—	—	—	—	0	0	48	0	—	—	—	—	—	0	0	0	0	0	0	—	—
" 17	0	0	0	9	∞	∞	6	—	—	24	4	∞	47	5	∞	—	—	—	0	0	0	48	0	—	—	—	—	0	0	0	0	0	0	—	—
" 18	0	0	9	42	∞	6	3	—	—	0	0	8	24	0	0	—	—	—	0	0	0	7	5	∞	—	—	—	0	0	0	0	0	0	—	—
" 19	0	1	0	4	∞	49	3	—	—	0	0	10	2	33	32	—	—	—	0	0	0	12	31	1	∞	∞	0	0	0	0	0	0	0	—	—
" 20	0	2	∞	4	∞	43	15	—	—	0	0	0	43	3	0	—	—	—	0	0	1	6	5	∞	10	—	0	0	0	0	19	55	∞	—	
" 21	—	4	0	14	∞	38	3	—	—	0	1	1	9	0	0	—	—	—	0	0	0	0	5	∞	—	—	0	0	0	0	10	0	0	—	—
" 22	0	0	8	6	∞	15	0	—	—	0	—	1	4	0	—	—	—	—	0	0	1	0	6	0	—	—	0	0	0	0	0	0	0	—	—
" 23	0	0	37	23	7	—	—	—	—	0	—	42	45	∞	0	—	—	—	0	0	1	0	—	—	—	—	0	0	0	0	0	0	0	—	—
" 24	0	0	0	1	∞	38	0	—	—	0	—	4	∞	∞	7	—	—	—	0	0	8	1	0	—	—	—	0	0	0	0	13	—	—	—	
" 25	0	0	0	1	∞	38	0	—	—	0	1	36	21	0	—	—	—	—	0	0	10	12	48	—	—	—	0	0	0	0	0	16	14	11	—
" 26	0	0	0	0	46	1	—	—	—	0	4	0	17	30	0	—	—	—	0	0	1	0	12	—	—	—	0	0	0	0	0	3	1	5	—
" 27	—	0	0	13	∞	∞	13	5	—	0	3	3	23	∞	25	—	—	—	0	0	1	6	0	—	—	—	0	0	1	15	15	—	—	—	
" 28	0	0	2	0	13	∞	27	30	—	—	0	0	0	∞	0	—	—	—	0	0	3	0	2	—	—	—	0	0	0	0	12	30	—	—	
" 29	0	0	0	0	45	∞	9	—	—	—	—	—	—	—	—	—	—	—	0	0	45	∞	—	—	—	—	0	0	0	0	1	28	—	—	
" 30	0	1	2	61	∞	32	5	—	—	—	—	—	—	—	—	—	—	—	0	0	4	40	15	—	—	—	—	—	—	—	—	—	—	—	
" 31	0	0	1	∞	9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Total no. of leaves	175					155					163					160					158					157									
Less I	33 (18.9 %)					17 (11.0 %)					26 (16.0 %)					7 (4.4 %)					5 (3.2 %)					2 (1.3 %)									
Less II	28 (14.8 %)					16 (10.3 %)					12 (7.4 %)					9 (5.6 %)					7 (4.4 %)					4 (2.5 %)									
Less III	15 (8.6 %)					26 (16.8 %)					17 (10.4 %)					11 (6.9 %)					15 (9.5 %)					12 (7.6 %)									
Less IV	41 (23.4 %)					33 (21.3 %)					30 (18.4 %)					19 (24.4 %)					31 (19.6 %)					20 (12.7 %)									
Total no. of leaves infected	115 (65.9 %)					92 (59.4 %)					85 (52.1 %)					38 (42.5 %)					58 (36.8 %)					38 (24.2 %)									



greater than in Exp. 1, corresponding with the slightly higher air temperature employed. This second experiment indicates also that the apparent decrease in infection at the highest humidity observed in the previous case was not significant.

Under constant conditions, therefore, it appears that infection occurs most readily when a high air temperature is coupled with a high humidity, and a decrease in either of these factors reduces the amount of disease. The methods of action of the two factors are, however, entirely different in nature. In the previous paper the theory was advanced that a high air temperature acts indirectly on the parasite by affecting the rate of maturation of the host, and possibly by altering its carbohydrate metabolism, with a resulting increase in the sugar content of the leaves. It is probable on the other hand that the importance of humidity is mainly physical in nature. The bacteria are motile, and gain access to the tissues of the leaf through the stomata. The method of inoculation adopted of spraying the leaves with an emulsion of the organisms deposits the bacteria in innumerable droplets of water on the leaf surface, and each droplet will cover a number of stomata. If the droplet persists until the bacteria have entered the stomata a lesion will result provided the other conditions are favourable. The average time the droplets will remain depends directly upon their size and upon the humidity of the atmosphere, so that the chances of infection occurring are greater with a high than with a low humidity. Even at low humidities, however, some drops of larger size will persist for a sufficiently long time for penetration of the stomata to be effected. Local variations in the humidity will also occur, and in some cases leaves will stick together while wet, with the result that some leaves become severely infected even at these low humidities. That humidity is of importance only up to the time when penetration of the stomata is achieved by the bacteria is shown by the fact that in experimental inoculations in the glasshouse, infection rarely occurs from spray inoculations, while a lesion invariably results from prick or injection inoculations. The problem of the time during which a high humidity must persist will be discussed in a later paper on the subject of alternating and regularly fluctuating conditions.

#### SUMMARY.

Experiments carried out in the Rothamsted control chambers on the influence of atmospheric humidity on the angular leaf-spot disease of cotton, resulting from spray inoculation of young plants, show that high humidities favour the development of the disease. Maximum infection

occurs at humidities exceeding 85 per cent. and at humidities below this the degree of infection decreases rapidly.

The relation of these results to the experiments on the influence of air temperature is discussed, and it is concluded that the importance of humidity is mainly physical in nature, by affecting the time during which the infection droplets persist.

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(Received January 15th, 1932.)

# EXPERIMENTS IN ENGLAND, WALES AND AUSTRALIA ON THE EFFECT OF LENGTH OF DAY ON VARIOUS CULTIVATED VARIETIES OF WHEAT

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(With Plates XXII and XXIII, 3 Graphs and 1 Text-figure.)

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## I. INTRODUCTION.

It has been known for some time that varieties of wheat which make satisfactory growth in Australia are failures in England, showing but poor development and giving very low yields. Further, they usually appear as very early varieties. Similarly in Australia, English varieties commonly fail to produce any satisfactory result; being prone to produce too many tillers and to develop their heads too late in the season to avoid the hot dry summer, characteristic of the wheat belt in the southern parts of Australia.

In 1928, Prof. Engledow transmitted to Australia six varieties which had been raised as hybrids at the Cambridge Plant Breeding Station, and with regard to three of them he stated that "They were early ripening hybrids and definitely earlier than any commercial varieties we have in England." Seed of these varieties was distributed to several centres in Australia, and although the actual growing period which they had was longer than would have been experienced by a spring wheat in England, yet they were later in heading than were the normal Australian varieties.

It seemed probable that this result might be largely determined by the length of day factor which has been shown to be of such importance in influencing time of flowering and ripening, and consequently field performance, in many plants. The pioneer investigations of Garner and Allard (15) have been confirmed and extended by many workers. Reviews of the literature on this subject have been written by Kellerman (26) and Redington (39) and render any general introductory discussion unnecessary. Further, a list of titles of papers dealing with this subject, compiled by one of the present authors, is available in a more recent report (see (47)).

It was decided to test this hypothesis by growing certain of the English varieties in Australia under conditions in which the length of illumination period could be altered to that of the normal day in England; and by growing certain Australian varieties in England.

In order to test the matter more fully, co-operation was established between the Royal Horticultural Society's Gardens at Wisley and the Welsh Plant Breeding Station at Aberystwyth on the one hand, and with the Victorian State Research Farm at Werribee (20 miles west of Melbourne) on the other. Dr M. A. H. Tincker undertook the work at Wisley and Mr T. J. Jenkin assisted him in planning the Aberystwyth experiments. The work at Werribee was conducted by Messrs H. C. Forster and A. J. Vasey, under the supervision of Professor S. M. Wadham. At each station other

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varieties of wheat were grown under similar experimental conditions for purposes of comparison, and various allied experiments were made in order to elucidate other matters. Daylight graphs for the latitudes concerned are based on data very kindly furnished by Dr J. M. Baldwin, the Government Astronomer of Victoria. They are to be found in Appendix I.

### *The varieties tested.*

For the sake of brevity, symbols have been used for these in the ensuing paragraphs. The varieties were:

41 C = F 8 W 41 C of the Cambridge Plant Breeding Station, a hybrid of Yeoman and Canberra (a fairly well-recognised Australian variety), which had proved to be the earliest of the original hybrids sent from Cambridge.

126 = F 6 W 126 of the Cambridge Plant Breeding Station, a hybrid of "Rhodesia" and "Dutch."

RM = *Red Marvel*.

FG = *Free Gallipoli*.

Re = *Ranee*.

Rj = *Rajah*.

Whilst at the British Stations two late varieties were also included:

H 1 = *Hen Gymro* pure line 490 } very late varieties produced at the Welsh Plant

H 2 = *Hen Gymro* pure line 274 } Breeding Station (for notes see Jenkin (24)).

## II. THE EXPERIMENTS IN AUSTRALIA IN 1929.

### (1) *Artificial modification of the daily light period.*

Varieties 41 C, 126, RM, FG, Re and Rj were used. They were sown in two sections; the first being under ordinary daylight throughout the experiment and the second under ordinary conditions until September 9th, when artificial illumination was added to bring the daily period of illumination up to that experienced by plants growing under conditions of cultivation at Wisley six months later.

Each section was sown in duplicate in the field at the rate of 90 lb. per acre and was manured with superphosphate at the rate of 4 cwt. per acre. Each section was also seeded at two different times:

(a) Early, sown May 31st: germinated June 13th.

(b) Late, sown July 5th: germinated July 22nd.

The plots were watered as necessary to avoid possible complications due to moisture deficiency. Owing to soil troubles the plants did not mature in a satisfactory manner; consequently data on the earlier stages of the plants' life are alone worthy of consideration.

There was no significant difference between the sections which differed in illumination only; and it became clear that the extra illumination had

been applied at too late a date in the life cycle of the plants to affect the behaviour prior to the "heading" stage; as has been already stated, the later part of the life history was upset by other factors. There were, however, considerable differences between early- and late-sown plots, shown in Table I.

Table I.

*Showing data from the early- and late-sown plots in the 1929 experiment at Werribee.*

	Early seeding	No. days	Late seeding	No. days
Seeding	May 31st	0	July 5th	0
Germination	June 13th	13	July 22nd	17
Jointing	Aug. 28th (approx.)	76	Sept. 25th (approx.)	65
<i>Heading:</i>				
Re	Oct. 11th	120	Oct. 26th	96
Rj	" 13th	122	" 27th	97
FG	" 27th	136	Nov. 4th	105
41 C	" 26th	135	" 5th	106
RM	Nov. 8th	148	" 13th	114
126	" 10th	150	" 14th	115

Av. soil temp. at 1 in.

June 1st-Oct. 30th, 51.4°. July 5th-Nov. 8th, 53.6°.

## (2) Fortnightly sowing observations.

In addition, a further experiment was also made by Messrs Forster and Vasey:

Short rows of four different varieties were sown at Werribee at periods of approximately 14 days' interval throughout the year. Periodical observations were made on the development of the plants in the rows. All plants showed much the same type of growth apart from their varying maturity. The following table, p. 382, gives results for FG as a typical example.

Graph I illustrates the data of Table II as far as the period from germination to heading is concerned. It is noteworthy that the peak of the curve of the number of days to "heading" lags behind the trough of the curve of "length of day" by a period of approximately 30 days. On the other hand the bottom of the trough of the former curve precedes the crest of the curve of "length of day" by about 10 days. The curve of average soil temperatures experienced by each group of plants shows quite clearly that the plants requiring a long period in which to mature experienced soil temperatures which fell below the general average during most of their development, whilst the opposite state of affairs prevailed in the case of those plants which had a short life cycle. It seemed reasonable to suppose that this factor, temperature, plays a significant part in

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modifying the effect of the length of the period of illumination on the time required for development.

Table II.

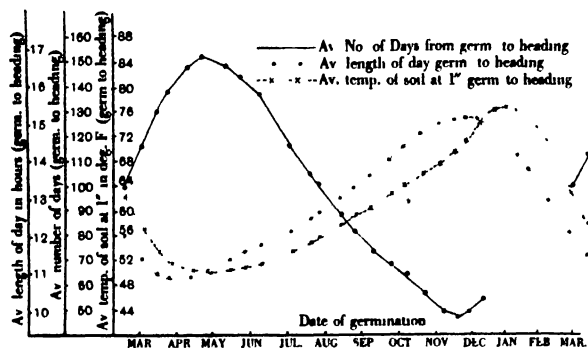
*Drawn from the fortnightly sowing experiment with FG at Werribee, 1929, and showing for each seeding the period of germination, and the periods from germination to jointing and to heading, also the average soil temperature and average length of day experienced by the plants of each group during the stages from germination to jointing and germination to heading.*

No. of days seeding to germination	Date of germination (g.) <sup>1</sup>	Jointing data			Heading data		
		No. of days g. to j.	Av. soil temp. at 1 in. ex- perienced in ° F.	Av. length of day experienced in hours	No. of days g. to h.	Av. soil temp. at 1 in. experienced in ° F.	Av. length of day experienced in hours
6	20. ii.	50	69.2	13.0	99	62.0	12.0
8	6. iii.	58	63.0	12.3	114	56.5	11.4
7	19. iii.	63	58.2	11.7	133	52.5	11.0
6	28. iii.	63	56.2	11.4	137	50.5	10.8
9	15. iv.	77	51.4	10.7	147	49.3	10.9
10	27. iv.	79	49.2	10.5	155	49.4	11.1
12	16. v.	75	46.6	10.3	146	49.8	11.3
10	31. v.	77	46.8	10.5	146	50.6	11.6
14	13. vi.	76	46.9	10.6	136	51.5	11.7
19	6. vii.	72	47.0	11.1	115	52.5	12.0
21	22. vii.	65	49.0	11.6	103	54.0	12.4
17	29. vii.	63	51.2	11.7	100	55.2	12.6
19	17. viii.	50	53.8	12.1	87	57.2	13.0
11	28. viii.	46	55.5	12.4	80	58.6	13.3
14	11. ix.	43	58.4	13.0	69	59.9	13.6
15	26. ix.	40	61.3	13.5	66	62.4	14.0
13	8. x.	37	62.5	13.8	63	63.8	14.4
11	22. x.	34	65.0	14.4	56	65.3	14.7
10	8. xi.	22	67.0	14.9	47	66.8	15.0
8	19. xi.	23	68.5	15.1	46	68.5	15.1
8	28. xi.	31	71.3	15.3	47	70.6	15.3
7	9. xii.	38	73.9	15.3	54	73.9	15.2

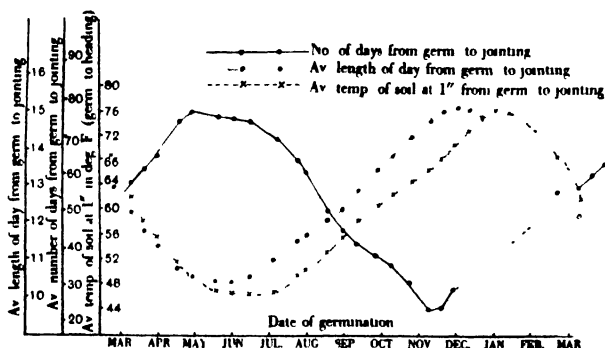
<sup>1</sup> It must be explained that date of germination, or brairding, means date of emergence of the coleoptila above the soil level. Date of jointing is the first date on which the axes of the plant obviously begin to show elongation, whilst date of heading is the date on which the ears first begin to emerge from the leaf sheath of the highest leaf.

It seemed desirable to subdivide the period between germination and heading by making observations on the "jointing" stage. The actual decision of date of jointing is necessarily somewhat vague, but the assumption of an almost erect habit by the tillers was found to be contemporaneous with a change of activity in the growing points which are pushing on with the development of the ear primordia at that time.

Possibly it is the outward morphological sign of the physiological change which has been noted in growth-rate curves by several observers (see Briggs, Kidd and West (6)).



Graph I. Drawn from data of the fortnightly seeding trial at Werribee, 1929, showing the relationship between date of germination of each set of plants, the average length of day and average soil temperature experienced by each group, and the length of period between germination and heading.



Graph II. Drawn from the data of fortnightly seeding trials at Werribee, 1929, showing the relationship between date of germination of each set of plants, the average length of day, and average soil temperature experienced by each group and the length of period between germination and "jointing."

The result of the subdivision of the period of development is given in Graph II, which shows that the curve of the number of days to "jointing" reaches a maximum just before the curve of the average length of day experienced reaches the bottom of its trough; the peak of the latter is reached about a fortnight earlier than the trough of the former. It is clear therefore that the factor, "length of illumination," modified by



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soil temperature, is operative during the stages of development prior to "jointing."

### III. THE EXPERIMENTS IN ENGLAND AND WALES IN 1929.

#### (1) *Methods.*

The plants were grown in pots in a medium loam. All the plants received an adequate supply of water throughout the entire period of their growth, so that there was no possibility of drought. Five seeds were sown in each pot (10 in. size), and after germination and the subsequent establishment of the seedlings the requisite thinning took place. Average representative plants remained at the rate of two in each pot, or where long 7 in. diameter glazed "culture pots" were used one plant remained. At least twelve, frequently twenty, plants constituted a series for each variety and each treatment.

In certain experiments the plants were grown at two centres, at the Royal Horticultural Society's Gardens, Wisley, and at the Welsh Plant Breeding Station, Aberystwyth; in other experiments only at the former station.

The following series were grown at Wisley:

- A. Controls receiving the full period of natural daylight, which varied approximately from 14.25 hours on April 10th to 17.25 hours on June 10th, to 16 hours on August 10th.
- B. Plants subject to 10 hours' daylight 7 a.m.-5 p.m. (G.M.T.), supplemented by electric light from 5 p.m.-11 p.m. of an intensity of 4-5 candle-power at the level of the soil.
- C. Plants receiving 10 hours' daylight (7 a.m.-5 p.m.).
- D. Plants receiving 6 hours' daylight (11 a.m.-5 p.m.).

And at Aberystwyth:

- A. Controls receiving the full period of natural daylight.
- C. Plants receiving 10 hours' daylight (7 a.m.-5 p.m.).
- D. Plants receiving 6 hours' daylight (11 a.m.-5 p.m.).
- E. Small field trials at the Welsh Plant Breeding Station's Farm. Sown in rows on the same date as A, B, and C.

The plants receiving the shortened periods of daylight were placed in the darkness of a hut specially constructed, so that an adequate supply of air circulated freely throughout the sections into which it was divided. The minimum temperature of the hut was on the average 2.5° F. higher than that of the outside air in May and June and on the average 1.55° F. higher in July and August. During spring and summer the effect of the

electric light installed in one section was to raise the minimum temperature by  $0.5-0.75^{\circ}$  F. above that of the unlighted sections. In winter, the effect of the light on the minimum temperature was too small to be observed. The greatest difference in temperature was therefore found between the control series, outside the hut, and the series receiving the electric light. All plants were exposed to the ordinary climatic conditions when not placed in the darkness of this hut, so that during the period (of photo-synthesis) in the daylight the temperature of all the plants was the same. In the morning when the plants of the 6-hour series were in the dark, a difference of temperature of approximately  $1.5^{\circ}$  F. was observed. The plants in the dark were now cooler.

The varieties Re, Rj, FG, 126, 41 C, H 1 and H 2 were tested.

Sowing of all the varieties including the winter ones took place at the beginning of April. The seed samples were obtained direct from the country of origin of the varieties. As full controls, short replicated rows were sown on the same date at Aberystwyth at a rate of sowing suitable for a medium tillering variety. The establishment of the plants in these rows was highly satisfactory, and throughout the season the plants were free from disease.

## (2) *Observations on the plants.*

### (a) *All varieties*<sup>1</sup>.

Some of the data collected at the two centres are shown in Table III. The figures shown are average figures for all the plants of one variety subjected to a stated treatment, and differences observed between treatments if as small as 2 days are of doubtful significance; differences as large as 4 days are certainly significant.

The order in which the varieties exerted spikes was the same under the control conditions at the two centres despite differences in temperature and other climatic factors. Despite their higher latitude, and therefore longer day, the plants grown in Cardiganshire in the cooler and moister environment required a longer period of time from brairding in which to flower than did those grown in Surrey, where the temperature rises more rapidly. The space enjoyed by the former plants was also slightly greater owing to the difference in the shape of pot used, a factor which would probably also tend to favour their vegetative growth. The behaviour of

<sup>1</sup> Preliminary notes on some of these results are to be found in the *Report of Conference of Empire Meteorologists London, 1929*, II, 34 (*Expt. Sta. Record*, LXII, No. 7, p. 612).

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plants in the field afforded confirmation of that observed in the pot cultures.

In general appearance and behaviour, the series B (10 hrs + 6 hrs) at Wisley resembled the control series A. The plants of the former produced visible spikes only a few days later than did the control plants A. The intervarietal order was the same in these two series. But plants of these two series A and B differed greatly from those subjected to 10 hours' daylight only (series C). The spikes of the latter appeared on the average for all the varieties almost a month later. Even more striking were the differences observed in vegetative growth: the plants of the 10-hour series, C, were obviously more "leafy<sup>1</sup>," and had produced a greater number of tillers than the plants of A or B series. The differences in appearance and behaviour between B and C series must be held to be due to the prolongation of the period of illumination despite the weak intensity.

In general appearance and behaviour during the first two months of growth the plants of series D resembled those of series C. More particularly was this so at Aberystwyth where the similarity existed far into the season and was apparent in August. At Wisley, as the season advanced the plants of D series produced less vegetative growth than did those of C series. At both centres the delay in the production of spikes in the D series was even greater than that caused by 10-hour periods of daylight.

The varieties that produced spikes (stamens and stigmas) "early" under the shortest period of light, were also "early" under the longer periods of light. The varietal sequence remained unchanged except in the relative position of 41 C which in all cases reacted differently from the others. The order in which the plants of the varieties exerted spikes was first, the Australian varieties; followed by the early English new hybrids; then the standard variety RM: the late winter varieties did not fully exert inflorescences producing pollen except under the conditions of the B series. Subjected to shorter periods of light the Australian varieties proved earlier, whereas the later varieties and winter forms did not flower.

In pot cultures unnatural development of the roots in relation to the depth of the soil takes place. For this reason no data upon root development have been presented. It may be briefly stated, however, that the observations made indicated that the very short periods of light (6 hours) (D), caused less root to be formed. The fibrous tufts of roots of the 10-hour series (C), the 10-hour and electric series (B), and the full-daylight

<sup>1</sup> "Leafy" = "flaggy."

Table III.

Giving data of the 1929 experiments at Wisley and Aberystwyth. *Wisley data are in roman type, Aberystwyth data in italic.*

Treatment	Variety	No. of tillers after		Days to emerge of spikes	Days to anthesis	Days to grain in milky dough stage	Days to yellowing of base of straw	No. of ears per plant
		42 days	63 days					
A (daylight)	Re	4.9	3.0	68	84	99	105	3.75
	Rj	6.3	3.0	69	85	100	105	3.75
	FG	4.5	4.0	73	85	102	107	3.5
	126	5.0	3.8	73	80	101	111	5.0
	RM	6.0	3.5	81	92	103	112	4.8
	41 C	16.5	7.0	89	94	104	110	4.8
	H 1	11.0	9.0	117	120*	—	—	(8)†
	H 2	12.0	11.6	126	—	—	—	(11)†
B (10-hour day plus 6 hours of 4 c.p. artificial light)	Re	5.0	—	70	87	98	—	3.75
	Rj	6.7	—	73	82	96	—	3.75
	FG	7.4	—	79	82	97	—	3.2
	126	8.3	—	115	91	103	—	5.2
	RM	6.9	—	116	107	113	—	4.2
	41 C	18.7	—	120	107	126	—	5.0
	H 1	19.2	—	131	133	—	—	(9)†
	H 2	19.6	—	136	—	—	—	(8)†
C (10-hour day)	Re	7.5	9.4	104	118	129	115	6.5
	Rj	8.6	9.6	107	120	141	155	5.2†
	FG	8.2	8.1	117	122	141	160	6.0
	126	14.7	9.5	139	162	167	182	14.0
	RM	10.5	8.5	143	165	176	182	5.0
	41 C	19.0	12.0	132	124	155	182	5.0
	H 1	18.0	14.8	—	—	—	—	0 (24)†
	H 2	19.0	19.8	—	—	—	—	(30)
D (6-hour day)	Re	5.7	7.0	139	161	Develop-ment	Dates not recorded	5.5§
	Rj	3.6	6.9	139	163	very poor or no grain	—	6.6§
	FG	5.7	7.9	147	171	—	—	4.0
	126	7.0	8.3	166	180	—	200§	6.8
	RM	5.5	10.3	171	Develop-ment	—	200‡	6.8
	41 C	8.2	13.0	167	very poor or none	—	—	4.0
	H 1	8.1	13.7	—	—	—	—	0 (25)
	H 2	8.2	10.6	—	—	—	—	0 (27)§§

Note. Sowing dates 4. iv. 29 and 7. iv. 29. "Days" = days from braiding to...

\* Did not ripen.

† Did not emerge.

‡ Green leaf 240 days, tendency to branch and leaf production.

§ Did not emerge in the normal manner, only tips of ears visible in a few tillers.

§§ No spikes out.

† Spikes did not emerge normally.

§ Green leaves 240 days.

‡ Some green leaf production 240 days.

§ Green leaf 240 days.

§§ No spikes emerged, "shot up" but not out.

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series (A) were of approximately the same size and density. The root development of the winter wheats with the longer period of vegetative growths was greater than that of the spring wheats amongst which varietal differences were not sufficiently large to be observed in the pot cultures employed.

#### (b) *Welsh Winter varieties, H 1 and H 2.*

The control plants (A) produced many erect tillers, from about a fifth of which spikes appeared. The exsertion was never completed, so that no ripe ears were produced. The plants of B series, which during late autumn enjoyed longer daily periods of illumination than the controls, produced pollen and a little ripe grain. The short days of autumn and winter prevented floral development in series A. These plants, however, overwintered, and in the subsequent summer spikes were produced from the majority of the surviving tillers of plants of both A and B series.

Under the shorter periods of light the vegetative period of growth was prolonged, and many, frequently more than seventy, side tillers were formed so that such plants grew to resemble in habit a caespitose grass. During the frost season no tillers became fully erect and no spikes appeared, but indications that the plants had completed the initial stages of floral development were available in that many tillers became semi-erect, and such tillers on dissection in winter usually contained an inflorescence arrested at an early stage of development. In these cases the length of stalk supporting the inflorescence from base of tiller to the lowest spikelet was about 7.5 mm., whilst the length of the glumes of the basal spikelet was about 1.5 mm., their width about 0.5 mm. There was little or no differentiation of individual flowers; the paleae were very small on the oldest florets. Many such tillers withered and did not survive the winter, others produced spikes early in the subsequent summer (June).

In the C series, the emergence of the spikes in the subsequent summer was exceedingly slow, frequently taking nearly a month. Such spikes were supported by stems partially developed during the previous season and they did not mature under these conditions of curtailed illumination. In addition to the surviving tillers, a few new ones were produced in the spring. The fate of these was also governed by the length of the period of light, for unless the plants were transferred to longer periods of light no spikes were formed. The majority of such thwarted plants died at the end of the second season. Thus, when prevented from flowering in the first season, plants of this winter variety can produce spikes in two

ways—by some tillers over-wintering and by the formation of new tillers, if subjected to long periods of light in the subsequent season.

(c) *English spring varieties*, 41 C, 126 and RM.

Plants of series A and B produced ripe grain. The long straw matured normally and the plants died in the early autumn. At both centres the spikes of plants receiving only 10 hours daylight of the variety 41 C emerged a month later than those of control plants. Similarly treated plants of the varieties 126 and RM produced spikes almost two months later than their respective control plants. The great delay in ear exsertion observed with these varieties was accompanied by an increase in the number of tillers produced by the treated plants (at Wisley shown in column 3 of Table III).

The ears of the plants of C series ripened very slowly during the autumn, the straw remained green and the quality of the grain obtained was decidedly poor. Generally the plants remained green and relatively leafy, vegetative growth was greatly prolonged. The plants of the D series did not produce any serviceable grain—a few spikes were produced but maturation did not proceed normally. Vegetative growth continued slowly throughout the entire summer and autumn.

A particularly interesting feature of this experiment was the relationship observed between floral and vegetative growth. With plants of the varieties RM and 41 C in the C series, the uppermost leaf of the flowering stem frequently remained green and continued to grow above the ears. Further new leaves were formed at the nodes of the flowering stem and were enclosed by the sheath of the uppermost leaf. The plants overwintered and in the subsequent spring and early summer a few spikes slowly emerged on the old stalks, such inflorescences having survived the winter in an early stage of development. Renewed vegetative vigour occurred during the spring.

Similarly plants of the variety 126 produced in the autumn small branches from the nodes of the inflorescence stalks, each branch developing several small leaves. In the subsequent spring these side branches elongated but produced no flowers. The external morphology of such cases was bizarre; for at the end of the second season two series of leaves were present, namely those enclosing the base of the flower stalk and those curious leaves developed upon the stalk at its nodes. The plants of this variety also exhibited renewed vegetative vigour in the spring by further tillering; for example, one plant from seed sown in April, 1929, possessed fifteen ears produced in October, 1930, and borne

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on long stems (length 65 cm.), the grain did not ripen under the 10-hour period of light.

The majority of the RM plants died at the end of the first season, but a few showed renewed vegetative vigour in the second spring.

No plant of any variety survived into the third season.

During the first two months of their growth the plants of the D series resembled in habit of growth those of the C series. Although vegetative growth was prolonged, the number of tillers formed by these plants was less than that of the plants of the series C. The few ears that slowly emerged were at least three months later than those of the A series. The grain did not ripen. Vegetative growth continued slowly throughout the autumn, and in the late summer of the subsequent season an occasional ear emerged but produced no ripe grain.

#### (d) *Australian varieties.*

These varieties were the earliest under all the conditions of the experiment. In the field they produced ears rapidly on very short straw. The behaviour of the varieties Re and Rj was precisely similar. FG proved to be slightly later, and was closely followed by the early English hybrid 126 under long periods of light. The period of vegetative growth was extremely short, and even the C and D series of plants of these three Australian varieties produced very few tillers in comparison with British varieties similarly treated.

Despite an adequate supply of water very rapid discoloration of the leaf and straw took place in A, C and D series. As the spikes emerged in early summer from the C series plants, their grain ripened during the warmer months; whilst plants of the D series produced ears and poor grain at Wisley, but at the cooler centre Aberystwyth, the grain did not ripen well.

The only survivors into a second season of the A, B and C series of plants were a few individuals of variety FG under treatment C; of these one produced ears in the second season. Several of the spikelets of this plant consisted of *eight* well-developed flowers from the stamens of which pollen was liberated. However, no mature grain was produced.

## IV. THE EXPERIMENTS IN AUSTRALIA IN 1930.

### (1) *Methods.*

The 1929 experiments at Werribee had emphasised the necessity of applying the modification to the period of illumination at a much earlier stage in the life history of the plants. Accordingly three series of treatments were devised.

I. Plants growing in an outside plot in daylight were given such extra periods of artificial illumination by electric light as were necessary to bring the total daily period of illumination up to 14 hours. The extra illumination was determined by a lumeter and varied in intensity from 4 to 5 ft. c.p. to 8 ft. c.p. according to the position of individual plants in relation to the source. This variation in intensity made no observable difference to the affected plants.

II. Control plants with normal conditions of daylight, the length of which is shown in the Appendix.

III. Plants covered by a light opaque box painted white on the outside. This was moved on and off the bed. Light had access to the plants between 9 a.m. and 3 p.m. daily. The crude nature of this experiment was recognised, but it was the best arrangement which circumstances allowed, and as results showed it gave indications of the effect of reduced periods of illumination.

The six varieties Re, Rj, FG, RM, 126 and 41 C were sown and the whole series was duplicated at two seeding rates, one at 90 lb. to the acre (the normal rate for the district) and the other at half that rate. Each section consisted of three rows 4 ft. long and planted 7 in. apart. Only 41 C, Re and FG were subjected to treatment III.

The whole series of plots were duplicated for observation. The rows were hand sown at the equivalent of the above rates of seeding, *e.g.* the section to be sown at 90 lb. per acre had the seeds placed at inch intervals along the row.

The plots were sown on May 22nd, accompanied by the application of superphosphate (22 per cent.  $P_2O_5$ ) at a rate equivalent to 2 cwt. per acre. All the seed germinated by June 5th, but it was not possible until the 18th to give the extra light to series I, nor reduce the light in series III. The plants at this time were in the two-leafed stage.

Frequent observations were made on the stage of growth of all series, and from time to time representative plants were pulled to ascertain the position of the developing head. Counts of tillers were made on July 29th and on September 2nd.

Since the observations of 1929 indicated that the modification of the light was particularly effective in hastening the early portion of the life history of the plant, the extra illumination was cut off from one section on August 25th when the plants were in the mid-jointing stage of development. The use of the dark box was, however, continued until the end of the experiment.



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### (2) *Observations.*

Table IV summarises the data. Germination and early growth were satisfactory in all rows, and tillers began to form about the end of June. About the middle of July, a definite difference in development was noticed. Plants of series I showed a more upright growth, whilst those of series II (normal day) exhibited a squat prostrate appearance but had more tillers. Series III plants (6-hour day) were lighter in colour, showed slower development and were decidedly more squat in habit than either of the above. Counts of tillers confirmed the observations. The retardation of the development of the individuals in series III which was shown both at this and later counts was due to the use of the dark box.

Table IV.

*Showing data from the Werribee experiments of 1930.*

Treatment	Variety	Av. shoots per plant on		Position of head of the plant in inches from base of plant			No. of days from germination to emergence from sheath of		Height at harvest in ft.
		29. vii.	1. ix.	29. vii.	25. viii.	2. ix.	Shot blade	Head	
Series I, 14-hour day	Re	3.7	3.8	Differentiation just begun	8	9-10	94	103	2.5-3.0
	Rj	3.6	3.6		6	9-10	96	108	2.5-3.0
	FG	3.7	3.2		4-6	6-7	98	110	3.0-3.5
	126	4.0	3.7		4-5	6-8	106	117	3.5-4.0
	RM	3.7	4.1		3-4	6-7	118	127	3.5-4.0
	41 C	6.8	6.4		2-3	4-5	108	117	3.0-3.5
Series II, Normal daylight	Re	5.7	6.2	Differentiation begun, heads smaller than in series I	2-3	4-5	108	118	2.5-3.0
	Rj	5.0	5.4		2-3	4-5	108	119	2.5-3.0
	FG	6.3	6.1		1-2	3	118	128	3.0-3.5
	126	6.1	6.0		1-2	2-3	129	145	3.5-4.0
	RM	6.1	6.3		1	1-2	129	148	3.5-4.0
	41 C	9.5	10.1		1	1	119	130	3.0-3.5
Series III, 6-hour day	FG	5.8	6.7	No differentiation of head	↓	Still	143	160	1.3-1.7
	Re	6.0	6.8		↓	below	129	146	
	41 C	7.5	7.8		↓	1	154	172	

By August 25th, the plants of series I were definitely taller, being about 15-18 in. high with a characteristically slender upright growth and a marked absence of bottom "flag." (They were not "leafy" at the base.)

Plants of series II were about 12-15 in. in height, and were of a more "flaggy"<sup>1</sup> nature. The big difference between varieties noticed in series I was not so apparent in the growth of plants of series II. Plants of series III were smaller still, only 6-9 in. high, and much lighter in colour. The degree of development of the ear showed characteristic differences as shown by the table.

<sup>1</sup> "Flaggy" = "leafy."

Heading in series I was first noticed in the early varieties, Re and Rj, about September 17th, when the plants were 24–27 in. in height. Plants of series II at about September 17th were slightly shorter, while those of series III were only about 9 in. high. The order in which the heads were observed was as follows: Re, Rj, FG, 126 and 41 C, and lastly RM, which did not exert spikes before October 11th.

The first heading in series II was not observed until October 2nd, when the plants were approximately the same height as those of series I. The order of maturity here indicated that both 126 and 41 C had responded differently. Whereas in series I they had both headed at approximately the same time (about 7 days later than FG), 41 C here produced a head only 2 days after FG; whilst 126, here very close in time of maturity to RM, was 15 days later than 41 C. This order of maturity showed reasonable agreement with that observed during 1929.

Plants of series III commenced to head about October 30th, but the heads were pale in colour and weak. The plants only reached 15 in. in height. Here, 41 C headed approximately 12 days later than FG.

With the help of added moisture, all the plants set grain and were ripening normally. Depredation by birds caused the loss of most of the grain, and the observations on the later stages of maturity have not been included.

There were two points worthy of mention at this stage. Firstly, it is the experience of workers in Australia that Rj is an earlier wheat than Re by a matter of 8–10 days, and yet as far as heading was concerned Re was earlier than Rj. Secondly, among the English varieties at the heading stage there were some outstanding individual plants which “headed” earlier than their neighbours. It seems possible that these varieties may not be completely “fixed” in their response to length of day. If this be so, they indicate a basis for natural selection aided by artificial selection by man, a selection that has produced the present-day Australian varieties from the old English importations.

(a) *Rate of seeding.*

The observations on the plots sown at the rate of 45 lb. per acre showed that in all cases the lighter seeding was responsible for a lateness of maturity extending to about 5 or 6 days in the case of the plots receiving 14 hours' light; in the plots receiving the normal length of day, the difference between the two seedings, although apparent, was not so marked.

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### (b) *Modifications.*

The plants of series I from which the extra light was discontinued from August onwards, showed no differences in time of heading when compared with the corresponding plants on which the treatment was continued till harvest. The change was made when the plants were about 15–18 in. in height and the position of the developing spike varied from 2 to 3 in. from the base of the plant in the case of the late varieties, to about 8 in. in the case of the Re plants. Thus since none of the plants of any variety showed any variation in the time of heading from that shown by corresponding plants which continued to receive the light, it is concluded that the "length of day" chiefly affects the development of the plants prior to the development of the ears within the sheath.

## V. THE EXPERIMENTS IN ENGLAND DURING THE SEASON 1929–30.

### A. *Spring sowing.*

The seed samples obtained for this experiment were harvested from crops grown at the Plant Breeding Institute, Cambridge, so that any possible effects of the edaphic and climatic factors, due to the source of origin of the seed sample, upon the growth of the seedlings were eliminated. Varieties Re, Rj, FG, 126 and 41 C were used.

Sowing of Australian and English spring varieties took place on February 19th, a much earlier date than was possible in 1929. The subsequent seedlings were subjected to the various treatments as soon as they appeared above the soil. The requisite thinning took place in March. The other cultural details were similar to those of 1929, so that this experiment served as a seasonal duplicate except for the date of sowing.

### (1) *General observations.*

During the first two months the differences in growth observed between the plants of the series A and C were insignificant, whilst the plants of series B were slightly taller and more advanced. The plants of series D were smaller than those of the other series, in that the stems and sheaths (measured to the uppermost ligule) and leaf blades were shorter.

In early May, three months after the seedlings had appeared through the soil, notable differences between the plants of the various series were apparent. The control plants of the varieties Re and FG, possessed on the average one tiller more than did the plants of the B series. The latter had received a longer period of light in the early months of growth<sup>1</sup>, and

<sup>1</sup> See graph in Appendix.

had passed into the phase of elongation. At this date the control plants of the varieties 126 and 41 C were of only semi-erect habit, whereas those of the same varieties in the B series were erect and had advanced further towards flowering.

The vegetative phase of growth of plants of all varieties in series C was prolonged, so that a greater number of tillers consisting of short sheaths and stems was recorded. These tillers varied in habit according to the variety, those of the Australian varieties were erect, those of the English varieties prostrate.

The general growth of the plants in series D was retarded.

As development progressed, the inter-varietal behaviour was similar to that observed in 1929. Again the Australian varieties were particularly "early" and produced spikes rapidly. Between the different series the relative "earliness" of the precocious varieties in the B series was maintained for longer, so that the spikes emerged a few days earlier than those of the control plants. In other varieties the plants of A series caught up those of B series, as development proceeded and the natural daylight lengthened.

Plants of C and D series were much later and behaved as those grown in 1929.

After the late production of ears by plants of the variety FG of D series, vegetative growth took place at the nodes of the flowering stems from which small branches appeared. The growth<sup>1</sup> of such branches resulted in the production of secondary elongating stems. Only one developed on each primary stem. The remarkable point about this secondary growth was the production of a further rudimentary ear. On dissection glumes were found 2 mm. long by 1 mm. broad, enclosing in the basal spikelets very small paleae.

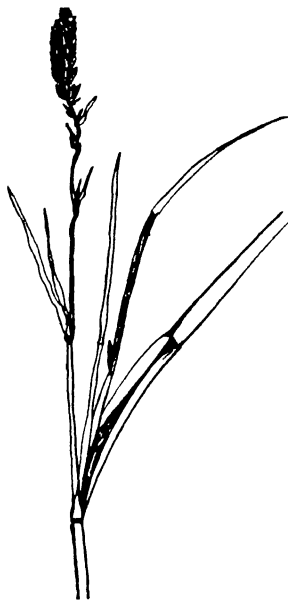


Diagram 1. Variety Free Gallipoli grown under a 6-hour daily period of light, showing vegetative branches from nodes of inflorescence stem. One such branch elongated to produce a sterile secondary spike. Reduced to  $\frac{1}{2}$  natural size.

<sup>1</sup> These abnormal growths resemble to some extent proliferations caused by pests such as *Phytophaga destructor*. A careful examination of the material was made by Mr G. Fox Wilson, Entomologist, Royal Horticultural Society. We are indebted to him for his report which states that no traces of such pest damage or of the pests themselves could be observed.

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The total length of these secondary ears never exceeded 3 cm. from the basal spikelet to the tip. The average size was 2.3 cm. (In the writers' experience, so far, no secondary inflorescence has produced functional flowers. This possibility might be realised by transference of such plants to other longer periods of light.) A diagram of the structure of these curious stems is on p. 395.

### (2) *Inter-seasonal and other comparisons.*

The date of sowing in the two years, Exp. 1, 1929, and Exp. 2, 1930, differed by about 6 weeks. The time taken by control plants of the Australian varieties to exert spikes was approximately equal in the two years. Similarly the date of emergence of the spikes was advanced by the early sowing in the case of plants receiving 10 hours' daylight and 6 hours' electric light. With the later English varieties a different response was obtained. The date of emergence of the spikes of control plants was advanced by only 4 days by sowing 6 weeks earlier.

The inference drawn from such observations was that the duration of the vegetative phase was prolonged by the shorter periods of daylight of early spring. The early Australian varieties were able to exert spikes under shorter periods of daylight, and were therefore not delayed by this factor.

That this is not the only factor<sup>1</sup> influencing the rate of floral development of the plants is clearly seen when the behaviour of the English varieties in series B was considered. Here the length of the period of light was of equal daily duration during the two seasons. The period of growth from brairding to full exertion of spike was longer in 1930 than in 1929. A delay in development occurred after the initial elongation had taken place; the precocity of the plants receiving the additional electric light observed during the "jointing" period was not visible after flowering.

The differences observed between the plants grown in 1929 and 1930 under periods of 10 hours' daylight were small, under the shortest period of 6 hours the seasonal differences were negligible.

### B. *Autumn sowing.*

One spring variety Re was sown together with the winter varieties H 1 and H 2 at the end of September. The seedlings were subjected to the various treatments as soon as they were visible. Owing to the short winter days certain modifications in the details of the light period were necessary. The following series were grown:

<sup>1</sup> See Gilbert (20).

$\alpha$  control. Full daylight 8.7 hours, November 30th. 8.6 hours, December 31st. 9.5 hours, February 1st. 10.9 hours, March 1st. 13.25 hours, April 1st.

$\beta$  series. Received during November, December, January and February all the available daylight (8–10 hours). In October and from April 10 hours' daylight, 7 a.m.–5 p.m. G.M.T. Throughout the entire period these plants also received 6 hours' electric light from dusk.

$\gamma$  series. During the winter months received all the available daylight. During the other months 10 hours (7 a.m.–5 p.m. G.M.T.).

$\delta$  series. Received 6 hours' daylight from (9 a.m.–3 p.m. G.M.T.).

The temperature conditions of series  $\beta$  were identical with those of  $\gamma$ . The electric light caused no appreciable raising of the minimum temperature. The series  $\alpha$  were exposed during the night to the full rigour of the climate. The automatic shelter given to  $\beta$  and  $\gamma$  by the shed caused the minimum temperature to be 1.5° F. higher than that outside on the very cold nights; at other times the difference was smaller.

#### (1) *General observations.*

Table V summarises the results. The winter was not a remarkably severe one, but all the plants of all varieties in the  $\delta$  series died during the winter months; the seedlings of the winter varieties survived slightly longer than those of the spring variety. This behaviour was a strong contrast to that observed with similar periods of light in spring and summer. It therefore appeared that the normal days of winter are so short as to be within some 2 hours of a period likely to cause entire failure.

All the other plants survived, including those of the early spring wheat Re.

In December and January it was observed that the leaf sheaths of plants of the variety Re in the  $\beta$  series were already longer than those of the  $\alpha$  or  $\gamma$  series. The plants possessed few branches, and generally their habit indicated further advance towards floral development. Similarly with the H 1 and H 2 wheats the additional electric light curtailed the development of tillers and resulted in the more rapid elongation of the leaf sheaths than that of control plants. Such observations were in agreement with those of the spring-sown experiments.

#### (2) *The Australian variety Ranee.*

The degree of floral development in the young inflorescences of plants of each series on May 20th is shown by data obtained by dissection<sup>1</sup> of the first shoot (= the main axis,  $T_0$ ) of each plant (see Table V, col. 9).

<sup>1</sup> We are indebted to Dr Hudson, Bureau of Genetics, Cambridge, for these data.

Table V.

*To show the response of plants of a winter and spring wheat grown during winter months to various periods of illumination. Date of sowing, September 29th, 1930.*

Series	Variety	No. of* tillers and habit, 3 months l. i. 30	Height to ligule of top leaf, 3 months cm.	No. of tillers and habit, 6-5 months 15. iv. 30	Height to ligule of top leaf, 6-5 months 15. iv. 30 cm.	Notes on earliest tillers, 7-25 months, 9. v. 30	Height to ligule of top leaf, 7-25 months 9. v. 30 cm.	Dissection of first tiller, 20. v. 30	Notes on earliest tillers, 8-75 months, 23. vi. 30
$\alpha$ Control. Short winter days. Full daylight	Re	2-6 semi-erect	3-5	2-5 erect	14-2	Erect, swollen high up	33-7	Stamens growing in first 3 florets.	Grain milky dough
	H 2	7-1 prostrate	2-8	8-8 semi-erect	3-0	Almost erect	12-0	7 florets developing	Pollen liberated
$\beta$ All available daylight up to 10 hours plus 6 hours' electric light	Re	2-0 erect	12-0	2-0 erect	24-4	All spikes exerted	56-2	Young grain developing.	Grain late dough
	H 2	6-5 erect	8-5	3-7 erect	10-3	Erect	22-4	7 florets developing. Larger than $\alpha$ series	Ears just emerged
$\gamma$ All available daylight up to 10 hours	Re	5-5 erect	5-1	3-6 erect	14-0	Erect, basal swelling	28-5	As $\alpha$ but stamens shorter.	Grain early dough
	H 2	9-6 prostrate	2-2	9-8 prostrate	4-0	Semi-erect	11-0	1 floret developing	Ears emerging

\* Only living tillers counted. A decrease in the number from one date to another is accounted for by the death of small tillers which did not produce spikes.

The plants of the  $\beta$  series maintained their lead and produced spikes, pollen, and grain at the "milky dough" stage at an earlier date than did the control ( $\alpha$  series) plants which were now receiving the longer periods of natural illumination. The grain of the  $\beta$  series was ripe early in July, but the control plants matured their grain rapidly and ripened it only 4 days later than the  $\alpha$  series. The plants of the  $\gamma$  series were at least 3 weeks later; they continued to develop green leaves until autumn, when they were destroyed.

(3) *The "late" Welsh varieties, Hen Gymro 1 and 2.*

The plants of the  $\beta$  series produced fewer tillers than did the control plants. The plants of  $\gamma$  series more closely resembled the controls in general habit of growth when examined in early spring. The early curtailment of vegetative growth was accompanied by early elongation of leaf sheaths and enclosed stems. The tillers of the  $\beta$  series became erect earlier than those of the control plants. Column 9 of Table V shows the development on May 20th.

From the beginning of June the control plants ( $\alpha$  series) received longer periods of light and developed rapidly, so that the relative earliness of the  $\beta$  series observed in May was not apparent by the middle of July. The plants of the  $\gamma$  series were much later in floral development, for although inflorescences were visible among them by the end of July, the rate of emergence of the spikes from the enclosing sheath was remarkably slow. The vegetative vigour of these plants ( $\gamma$  series) was prolonged, and in September their green "leafy" appearance afforded a striking contrast to the yellow and dry straw of the plants of  $\alpha$  and  $\beta$  series. No ripe grain was produced by these plants of  $\gamma$  series. Frequently the paleae and glumes enlarged and remained green, so that they resembled small membranous leaves. The total number of side tillers formed in many cases exceeded 50, and several plants produced more than 20 erect, but short, flowering stems. The plants when destroyed in October were still green and the leaf development active.

C. *Transference of plants from one daily period of illumination to another.*

(1) *Transference from short to long daily periods.*

(a) *Winter varieties—autumn sown*—H 1. The transference of prostrate plants with many tillers from the 10-hour periods of daylight to the full daylight of late May and early June caused the tillers to become erect quickly. This difference in habit was observed in 15 days' time. After



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7 weeks in summer there was no large difference in general habit or stage of ripening between transferred plants and others which had grown under the longer periods of light all the season. A difference remained in the number of ears produced.

(b) *English spring varieties—spring sown.* Similarly the transference of plants of the varieties 41 C, 126 and RM, from short to long periods of light accelerated floral development which was observed in 3 weeks' time. The interval necessary for transferred plants to attain the same stage of development as plants grown all the season under the longer periods of light was shorter than that required by the winter varieties.

(c) *Australian varieties—spring sown.* The transference of plants of the Australian varieties Re, Rj and FG from short to long periods of light resulted in an acceleration of the rate of elongation of the leaf sheaths and enclosed stems. In less than 3 weeks the smaller difference between plants transferred and the other plants which had received long periods of light throughout the season was no longer visible. The effects of the transference were visible in 14 days. On transferring plants from 6 hours' daylight to the long days of summer a similar acceleration of floral development resulted, but an interval of 9 weeks elapsed before these plants attained the same habit as the ripening control plants.

### (2) *Transference from long to short daily periods.*

(a) *Winter varieties—autumn sown—H 1.* The transference of plants from long to 10-hour or 6-hour periods of light checked the development of the flowering stems so that no spikes were exerted. On dissection a stem supporting an inflorescence was found in almost every erect tiller, the average length<sup>1</sup> of which was 15 cm. when transferred and 17.5 cm. when elongation ceased in 14 days.

(b) *English spring varieties—spring sown.* The rate at which elongation of the stem took place was retarded by transference from long to short periods of light. The plants when transferred had already produced erect tillers.

(c) *Australian varieties—spring sown.* When plants of Re, Rj and FG in the late tillering stage were transferred from long to short periods of light the rate of elongation of the stem was retarded and emergence of the spikes took place about 5 days later than from plants not transferred. Such transferred plants were very much earlier than others grown throughout the season in the shorter periods of light.

<sup>1</sup> Overall length from base of stem to tip of ear.

## VI. DISCUSSION OF RESULTS.

(1) *Comparison with other Gramineae.*

The results of these experiments confirm the fact that the wheat varieties fall into the category of "long-day plants" defined by Garner and Allard (15). The results are thus in agreement with those of Wanser (51) and Doroshenko (10) amongst others. Yoshii (54), on the other hand, reported that certain Japanese varieties of wheat under the conditions of his experiments flowered early with short periods of illumination. As has been here observed in these detailed experiments with wheat, varieties differ markedly in their response, and there is no sharp dividing line between long- and short-day plants. Under any set of conditions otherwise comparable there are probably upper and lower limits to the daily period in which any variety can produce flowers. The results confirm Wanser's (51) observations that the winter varieties are characterised by the fact that they continue to grow in a vegetative manner under short periods of light at cool temperatures. The other cereals previously tested by one of the authors (45) were oats and barley, pure lines of which gave similar results. The observed varietal differences have a counterpart in the differences exhibited by late and early strains of *Dactylis glomerata*, *Alopecurus pratensis*, *Lolium perenne* and *Phleum pratense*, in the response to this factor.

(2) *Growth in length and floral development.*

The weak additional electric light which proved effective in influencing the habit of growth can scarcely cause directly a significant increase in the products of photosynthesis. All the data obtained from these and other similar experiments indicate that this factor influences the rate of elongation and growth in length of the plants by some other means.

In their report of experiments in which certain varieties of spring wheat proved particularly responsive to long periods of light, Garner and Allard (17) described the influence of this factor upon tillering, date of exertion of spikes, length of straw and maturity. With them certain spring varieties (not tested in the present experiments) did not grow as tall under long periods of light as the more slowly maturing control plants. In the English experiments with winter varieties the rate of elongation with long periods of light was very much greater than under short periods. With spring varieties the plants receiving long periods of light grew taller than those subjected to short periods. The Australian

varieties which matured rapidly generally produced short straw, particularly when they were under the long periods of light. The straw of the plants receiving only 10 hours' light was longer. It seems reasonable to suggest that the final height of the plant is influenced by the rate of elongation and also by the length of the period from "jointing" (tiller erection) to maturity, and depends therefore on the reaction of any given variety in both these ways. A particularly critical stage in development influenced by the length of day, seems to be the end of the tillering stage of growth, from which follows the initiation of the process of stem elongation. During this stage the inflorescence commences to form; its development in the erect tiller may, however, be checked by transference to short periods of light, and floral differentiation may then be prevented; particularly may this occur in "late" spring varieties.

### (3) *The formative effect—floral development.*

That the effect of the length of day at the critical stage of growth is a formative one is stated by Hurd Karrer<sup>(25)</sup> in a recent publication. She grew plants in glasshouses, the temperature of which was controlled. Two intensities of electric light were employed, namely 50–100 c.p. and 15 c.p. At relatively low temperatures and under long periods of illumination a winter wheat (Turkey) left the rosette habit early and became erect. Presumably inflorescences were formed in such tillers, but dissections are not reported. At higher temperatures (20° C.) this wheat did not continue in the rosette habit even under short days. Hurd Karrer states that with winter wheat "At the more favourable temperature for normal growth (10–12° C.) the resting stage was produced by 8 and 9½ hours' natural light periods, but prevented by a 16½ hours' light period." The phenomenon of true dormancy was not observed in our English experiments; at lower temperatures than 10° C. slow vegetative growth continued. When floral development under short periods of light did not proceed, vegetative growth continued slowly in winter, or more quickly in spring and summer. *Short periods of light did not "produce a resting stage" at all.* We disagree with the quoted statement.

### (4) *Vegetative and reproductive growth.*

The data from the present experiments show that the length of day influences the relationship between vegetative and reproductive growth. Vegetative development, of which the number of tillers frequently provides an index, was prolonged by short days. Leaf development was

continued far into the season, and in certain cases it was continued even after flowering and ripening of the grain, in a manner comparable to the aftermath growth of grasses. Occasionally unusual morphological structures resulted by the production of leaves in unusual situations.

In this connection the work of Maximov (31, 33) and his associates on the length of the vegetative period of growth deserves close attention. His results with cereals show that where floral development was inhibited or retarded vegetative growth continued. With barley he found that vegetative development as measured by the number and size of leaves was greatly increased under short days (9 hours and 12 hours), whilst with millet the reverse was true. Long days prolonged vegetative growth, short days caused rapid floral development. Similar relationships were observed with other species such as *Phaseolus multiflorus*.

Our observations show that there is a connection between the length of the period of light and the longevity of the plant. Wheat plants prevented from flowering may grow for two years, or possibly longer.

The fact that under Victorian conditions "length of day" exerts a far greater influence on the early stages of development than on the later is a matter of very considerable importance. This is possibly correlated with the higher soil temperatures which obtain during the earlier periods of growth.

#### (5) *Varietal characteristics and source of origin.*

The Australian varieties tested proved capable of exerting spikes under shorter periods of light than did the later flowering British spring varieties. The length of day in Victoria does not exceed 15.5 hours, whereas in England it may be of 18 hours' duration. The comparative failure and general unsuitability of the British varieties for Australian conditions may be *partly* explained by these experimental results, for varietal success is an expression of adaptation to the environment. Such adaptation forms one of the problems confronting geneticists breeding new varieties. In making selections from old land varieties for further local use plant breeders have by their empirical methods, which pay attention to lateness and earliness, selected strains or built up "pure lines" suited for the given locality. Such selection has also been automatically carried out by cultivators whose methods tend to reject both particularly early and late strains, which may have arisen as "sports" or as the result of inter-varietal or other hybridisation. In this connection the Australian observation that certain plants in the English varieties were definitely earlier than the remainder may be of consider-

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able significance. These varieties were "pure lines" and had been carefully examined for purity for several seasons. It may be that this minor difference in character was, under English conditions, incapable of expression to a degree which could be detected.

It is not surprising to find that the source of origin of a variety is related to its photoperiodic response. Doroshenko (10) tested in northern latitudes a large number of cultivated cereals collected from widely separated origins. His results demonstrated that varieties from latitudes in which the days are short and do not vary greatly in length, are not retarded by artificial short periods of light, as are those from high latitudes. In a further series of experiments Doroshenko and Rasimov (11) studied the inter-relationship between vegetative and reproductive growth and the influence of the period of light upon the organisation of the leaves. The extended tests included varieties of wheat, barley, oats and millet.

Similar evidence has been collected from other agricultural crops by those who have studied the differences observed between strains from different sources of origin. For example, Stapledon (43, 44), working with *Dactylis glomerata*, reported that strains of this species from high northern and also from high southern latitudes are particularly "leafy," late flowering, and persistent when compared with strains from latitudes nearer the equator. The New Zealand strains resembled the British (from which they may have arisen), but formed contrasts in habit to those from southern Europe. Precisely similar observations with other species of grasses have been made (see (42)).

Other crops studied include red clover (*Trifolium pratense*). Williams (53), from his large collection of varieties and strains, concluded that those imported from southern latitudes were, relative to some indigenous and cultivated strains, generally earlier to flower, relatively poor in vegetative development, and short lived.

With wild plants Turesson (49) has collected ecotypes of common species, and on growing them together observed differences in habit similar to the differences of varieties of cereals or strains of grasses and herbage crops. It would seem that local adaptation is not a phenomenon confined to cultivated species. Of course there are significant differences in habitats as judged by the growth of the ecotypes, which are not related to the length of day factor but are edaphic. Further experimental tests are desirable so that the physiological characteristics of these ecotypes may be more fully explored.

(6) *Utilisation of products of photosynthesis, etc.*

Klebs<sup>(27)</sup>, working with *Sempervivum*, laid emphasis upon the total quantity of light and the consequent accumulation of carbohydrates received by the plant as a factor influencing the date of flowering. Garner and Allard<sup>(15)</sup>, Adams<sup>(1)</sup>, McClelland<sup>(34)</sup> and others have considered the utilisation of the carbohydrates in the explanations of their results obtained by altering the daily period of light. The carbohydrate/nitrogen hypothesis of Kraus and Kraybill<sup>(29)</sup> has been considered elsewhere by one of the authors<sup>(46)</sup> in this connection. Nightingale's<sup>(37)</sup> analyses of plant tissues afford further evidence in support of this theory.

Hicks<sup>(22)</sup>, working with wheat, demonstrated that as the plant reaches the flowering condition this ratio rises. Young and other vegetative tillers have been shown by Engledow and Wadham<sup>(14)</sup> to possess a high nitrogen content. Therefore in early varieties where few tillers are formed the ratio possibly rises more rapidly than in late-flowering varieties or those in which many new growing centres serve as a drain upon the supply of carbohydrates. Evidence exists, with oats, to show that the removal of such sources of expenditure of carbohydrates can accelerate the development of the remaining main axis (Tincker and Jones<sup>(48)</sup>).

Turning to the nitrogen divisor of the ratio, Weaver<sup>(52)</sup> traced out the differences in root development of a winter wheat, Kanred, and a spring wheat, Marquis. His diagrams show that the root development of the winter wheat at 55 days from sowing when tillering had taken place, exceeded that of the spring wheat at 60 days when elongation of the four tillers had already begun. Assuming an equality in relative efficiency of the roots and a similar distribution of root hairs, etc., the winter wheat would tend to have a lower C/N ratio at this "time age," and to be in a younger physiological condition. The development of such a root system must also utilise carbohydrates and so keep the ratio low.

Popp<sup>(38)</sup> demonstrated the importance of the quality of the light under which plants are grown; by eliminating rays below  $529\mu\mu$ , many pathological symptoms were evident. The blue violet rays are indispensable for normal growth. His cultures did not, however, include wheat but species of *Holcus* were tested. Shirley<sup>(41)</sup>, working with a number of species, reported that the dry weight, a large proportion of which is due to the carbohydrate fraction, increased directly as the intensity. With wheat Davis and Hoagland<sup>(8)</sup> similarly observed that the dry weight increased as the intensity of illumination. Their data suggest that the relationship between the length of day and the dry weight was an exponential one.

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Arthur (5) and his associates, by means of chemical analyses of plants grown under various periods of light, with and without additional carbon dioxide, and with controlled nitrogenous fertilisers, studied the relationship between the C/N ratio and the behaviour of the plants. A wide variation in this ratio was observed in radish and lettuce, long-day plants, and in buckwheat, an ever-blooming type, and also in *Salvia*, a short-day plant capable of regulating closely the percentage of nitrogenous compounds in the tissues. The magnitude of the ratio was not correlated with the flowering response of the plants. The length of day, however, was a deciding factor. Arthur concluded that this factor operates by some other mechanism than the C/N ratio, and citing the work of Knott (28) and others on the localisation of the response to a few apical cells suggests that a study of the enzymes or other substances present in small amounts in the growing tips offers more promise than gross carbohydrate and nitrogen fractions in elucidating the mechanism of flowering response. Yet it does remain true in certain species, as Arthur's own data with red clover show, that concomitantly with the production of flowers an increase in carbohydrates (and weight) takes place under long days. Arthur and his co-workers found that winter wheat, hybrid 128, showed a tendency to head when grown with daylight plus 6 hours of light, with additional carbon dioxide, whereas it did not do so under shorter natural illumination; another winter variety, Turkey Red, did not head under any of their experimental conditions. A spring variety, Blue Stem, headed especially well with additional light and carbon dioxide, in fact within a month from sowing spikes were visible. This work of Arthur's (5), under carefully controlled conditions, further emphasises the differential physiological response of varieties.

Lubimenko (30) has suggested that the fundamental difference between plants which react in different ways to the length of the period of light is to be found in the relative magnitude of the processes of respiration and assimilation. Similarly Eaton (12), who worked with Soy Bean, a plant particularly susceptible to changes in the length of day, considered that such factors as temperature and the length of day influence the ratio of carbohydrates produced and carbohydrates oxidised. These experiments and their interpretation are also concerned with the utilisation of the products of assimilation.

Alternatively, if we accept the view that for each variety a certain critical ratio has to be reached before primordia formation begins, then the difference between varieties might be to some extent explained by different critical ratios.

(7) *Economic aspects and applications.*

In conclusion the practical applications may be mentioned. By lengthening the daily period of light by artificial means Harrington (21) has facilitated the breeding of cereals by obtaining two generations in one year. At the Research Farm at Werribee this is now an accepted practice in certain cases during the early years of a new cross.

Emerson (13), by controlling the period of light, was enabled to cross *Reana luxurians* with *Zea Mays*. Similarly Davies (7), by controlling the time of panicle exertion, was enabled to cross late and early varieties of *Dactylis glomerata*, and the same principle is applicable to cereal varieties within a species.

Such experimental methods may materially assist in elucidating the physiological characteristics of varieties and genotypes, so that it may be possible to arrange and classify varieties upon a basis of performance in response to environment as well as upon their external morphological character. Much of the work of the plant breeder and agronomist is concerned with such varietal response.

As Wanser (51) has previously pointed out, as a result of his work with wheat, such experiments are of value in affording data relative to acclimatisation. In the importation of new forms for commercial purposes greater success would be obtained by the consideration of latitude in addition to meteorological factors.

## VII. SUMMARY.

1. This paper reports the results of experiments carried out in Surrey and Cardiganshire (lat.  $51^{\circ} 19' N.$  and  $52^{\circ} 25' N.$ ) and at Werribee in Victoria (lat.  $37^{\circ} 54' S.$ ) designed to test some Australian and British wheats grown under various periods of illumination. Light of weak intensity (4-5 c.p.) was employed to prolong the daily period.

2. Generally the Australian varieties were "earlier" at all centres under all periods of light than the British.

3. A sharp distinction between the reaction of spring and winter varieties was observed under short periods of light.

4. Retardation or omission of floral development was always accompanied by prolonged vegetative vigour. The influence of such treatments upon the longevity of the plants was observed.

5. Observations are made upon the relationship of the photoperiodic response to the geographic source of varieties.



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6. The results are very briefly considered from the standpoint of the C/N hypothesis and the utilisation of the products of photosynthesis.

7. Certain economic aspects of the work are indicated.

### ACKNOWLEDGMENTS.

It is the authors' very pleasant duty to express their thanks to Mr F. J. Chittenden, of the Royal Horticultural Society, and to Prof. R. G. Stapledon, Director of the Welsh Plant Breeding Station, for placing the facilities of these institutions at our disposal, particularly when it is remembered that the primary interests of these institutes are more concerned with other plants.

To Mr G. Fox Wilson, Entomologist, the authors express their thanks for the careful examination he made of the abnormal material submitted, and to Mr N. K. Gould for the excellent photographs.

To Prof. F. L. Engledow, Cambridge, for his co-operation and for the seed samples of new hybrids, to Mr T. J. Jenkin, who kindly sent samples of his pure lines of Hen Gymro wheat and who assisted in planning the Aberystwyth experiments, the authors are deeply indebted. Mr A. R. Beddows greatly facilitated the conduct of the experiments by assisting in note-taking at Aberystwyth, and the authors desire to thank him and Dr Hudson of the Bureau of Plant Genetics who kindly visited some of the cultures and by his experienced technique in dissection of very young tillers provided data of floral development.

Fortunately similar work is to be continued at the Plant Breeding Institute, Cambridge, by Dr Hudson.

We desire to thank Dr J. M. Baldwin, Government Astronomer, Victoria, for kindly furnishing us with seasonal data of the length of day.

The experiments were carefully carried out by the staff of the institutions, to whom we here express our thanks.

### APPENDIX.

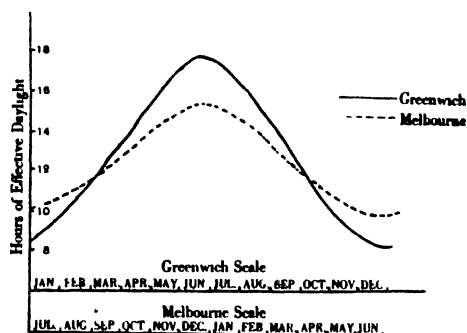
#### *The respective lengths of day at Melbourne and Greenwich for various days of the year.*

Previous workers along this line of research have used an illumination of from 3-5 ft. c.p., which may be taken as the intensity necessary for influencing plant growth in habit (cf. Garner and Allard (16)). This degree of illumination occurs when the sun is at a zenith distance of approximately 94° either before sunrise or after sunset.

Since the length of day at any given place may be presumed to be the length of time between these two points of the sun's passage across the meridian, the following table gives these data for Melbourne and Greenwich respectively:

Day of the year	Sun's declination °	Length of day at	
		Melbourne hr. min.	Greenwich hr. min.
Jan. 1st	- 23.0	15 22	8 40
" 16th	- 21.0	15 6	9 6
Feb. 1st	- 17.2	14 36	9 50
" 16th	- 12.4	14 2	10 42
Mar. 1st	- 7.6	13 28	11 32
" 16th	- 1.8	12 52	12 34
April 1st	+ 4.5	12 14	13 38
" 16th	+ 10.0	11 38	14 40
May 1st	+ 15.0	11 6	15 40
" 16th	+ 19.0	10 42	16 36
June 1st	+ 22.0	10 18	17 22
" 16th	+ 23.4	10 8	17 42
July 1st	+ 23.1	10 10	17 40
" 16th	+ 21.4	10 22	17 12
Aug. 1st	+ 18.1	10 44	16 24
" 16th	+ 13.8	11 14	15 26
Sept. 1st	+ 8.4	11 48	14 22
" 16th	+ 2.7	12 24	13 30
Oct. 1st	- 3.1	13 0	12 20
" 16th	- 8.8	13 36	11 20
Nov. 1st	- 14.4	14 16	10 20
" 16th	- 18.7	14 48	9 32
Dec. 1st	- 21.8	15 12	8 56
" 16th	- 23.3	15 24	8 36

Graph III which follows shows the relative periods for Wisley and Werribee with abscissae adjusted so as to bring out the comparison.



Graph III. Showing seasonal variation in length of day Melbourne and Greenwich.

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### EXPLANATION OF PLATES XXII, XXIII.

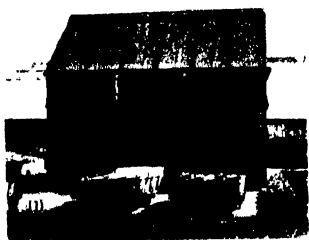
#### PLATE XXII.

- Phot. 1. The specially constructed hut showing the trucks on which plants were placed. Note the large louvres around all the sides on the doors and roof to facilitate ventilation. Electric light and time switch are in one end section. Charts for thermometer readings are also visible. Wisley. A similar technique was employed at Aberystwyth.
- Phot. 2. Typical plants from seed sown 19. ii. 29, receiving 10 hours' daylight and 6 hours' electric light photographed 1. vii. 30. Left to right: (1) Red Marvel; (2) Cambridge Hybrid F 8 W 41 C; (3) F 6 W 126. General similarity of response in English spring sown varieties. (Wisley.)
- Phot. 3. Typical plants from seed sown 19. ii. 29, receiving 10 hours' daylight and 6 hours' electric light photographed 1. vii. 30. Left to right: (1) Red Marvel; (4) Free Gallipoli; (5) Rancee. Note the "earliness" of Australian varieties (4 and 5) in contrast with Red Marvel. (Wisley.)
- Phot. 4. Plants from seed sown 17. ii. 30. Photographed 1. vii. 30. Left to right: (1) Red Marvel—10 hours' daylight; (6) Rajah—10 hours' daylight; (3) Hen Gymro—10 hours' daylight. Note that Australian variety (6) under short periods of light is early. The winter variety (8) has a caespitose habit (only two plants in pot) and is particularly late. (Wisley.)

#### PLATE XXIII.

- Phot. 5. Tillers of variety Cambridge hybrid W 126 plants receiving 10 hours' daylight. Seed sown 19. ii. 29. Photographed 17. viii. 29. Note left to right: continued leaf development on rachis bearing spike; centre, vegetative branches from nodes; right, elongation of branches arising from rachis. (Wisley.)
- Phot. 6. Young spikes of Rancee dissected out. A, 14-hour day—i.e. daylight and electric light. B, normal daylight. C, 6 hours' daylight. Sown 22. v. 30, photographed 25. viii. 30. (Werribee.)
- Phot. 7. Young spikes of Free Gallipoli dissected out. A, 14 hours' day—i.e. daylight and electric light. B, normal daylight. C, 6 hours' daylight. Sown 25. v. 30. Photographed 25. viii. 30. (Werribee.)
- Phot. 8. Young spikes of Cambridge Hybrid F 8 W 41 C. A, 14 hours' day—i.e. daylight and electric light. B, normal daylight. C, 6 hours' daylight. Sown 22. v. 30. Photographed 25. viii. 30. (Werribee.)

(Received December 15th, 1931.)



Phot. 1.



Phot. 2.



Phot. 3.



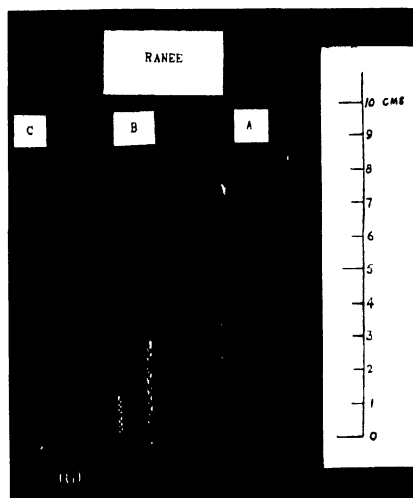
Phot. 4.

FORSTER, TINCKER, VASEY AND WADHAM. EXPERIMENTS IN ENGLAND, WALES AND AUSTRALIA ON THE EFFECT OF LENGTH OF DAY ON VARIOUS CULTIVATED VARIETIES OF WHEAT (pp. 378-412).

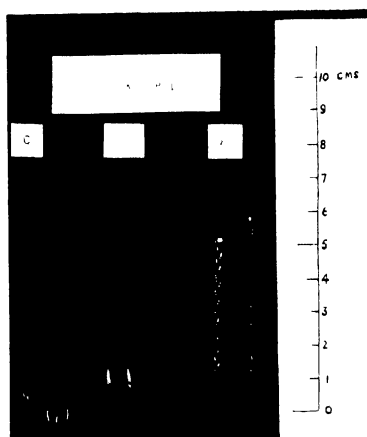




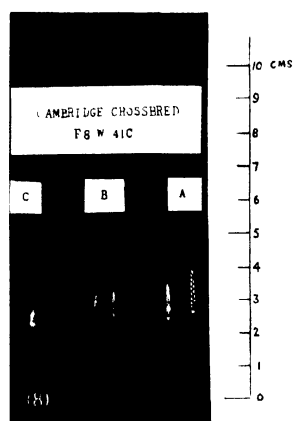
Phot. 5.



Phot. 6.



Phot. 7.



Phot. 8.





# ON A SUGGESTED METHOD FOR DETERMINING THE NUMBER OF LARVAL INSTARS IN *SITODREPA PANICEA* L.

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(*Fellow of the University of Wales.*)

(With 1 Text-figure.)

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## I. INTRODUCTION.

THE measurement of the rate of growth in insect larvae is a subject which has attracted increasing attention in recent years. As a result of the study of twenty-eight species of Lepidoptera, Dyar (1890) came to the conclusion that the rate of growth in successive instars, as measured by the increasing size of the head capsule, was in regular geometric progression. Fisher's work on *Tortrix pronubana* Hb. (1924) confirmed this conclusion. Miles (1931), working on four species of Tenthredinidae, finds that the width of the head capsule, or of the frons, in successive instars follows a regular geometrical progression in the initial stages of growth, but that in the later instars growth becomes irregular owing to sex differentiation and the occurrence of the prepupal stadium. Taylor (1931), also working with a Tenthredinid larva, *Phyllotoma nemorata*, concludes that, while Dyar's rule may continue to be useful in certain groups, it cannot be employed without reservation, and direct observation is the most satisfactory method of determining the number of instars in a given larva.

For the present investigation, *Sitodrepa panicea* L., an Anobiid beetle destructive to stored and dry goods, was selected, since larvae were readily obtained in large numbers, and are provided with well-developed head capsules.

## II. MATERIAL AND METHODS.

In growth studies, the width of the head capsule is usually selected as the unit of measurement, since it shows little or no increase in size during a given instar. The head capsules of Lepidoptera are shed whole at ecdysis, and Fisher was thus able to measure the cast skins of *Tortrix pronubana*. Miles found that the cast head capsules of Tenthredinid larvae, which split on being shed, are unsuitable for measurement. He was therefore obliged to kill the larvae before examination. The advantages of Fisher's method are obvious, since the same larvae can be measured in successive instars. Miles used a fairly large population of larvae, killing a definite number during successive instars and calculating the average width for each. From these calculations he was able to arrive at a growth ratio.

In the case of *Sitodrepa panicea* L., it was found necessary to adopt a different method of investigation. The larvae for this study were taken from cattle cake into which they had burrowed deeply. Cast skins of successive instars were difficult to obtain, since, once removed from their habitat, the larvae were difficult to rear. It was therefore decided to measure large numbers of the larvae over a given period of time and to examine the results yielded by such samples. Accordingly, samples of larvae were removed from the cattle cake between October and December, 1927, killed in 70 per cent. alcohol, and measurements made of the head capsule at its widest region. Unfortunately the measurements of these samples were not kept separate and are therefore taken to represent a single large sample. The series of measurements, totalling 887 larvae, is given in Table I.

It was to be expected that the measurements would reveal a few well-defined instars, a small range of variation in size (if any) in each instar, with possibly numerical differences in each instar due to the stage of development reached by the larvae at the time of sampling. Graph I, which is based on Table I, shows, however, that this is not so.

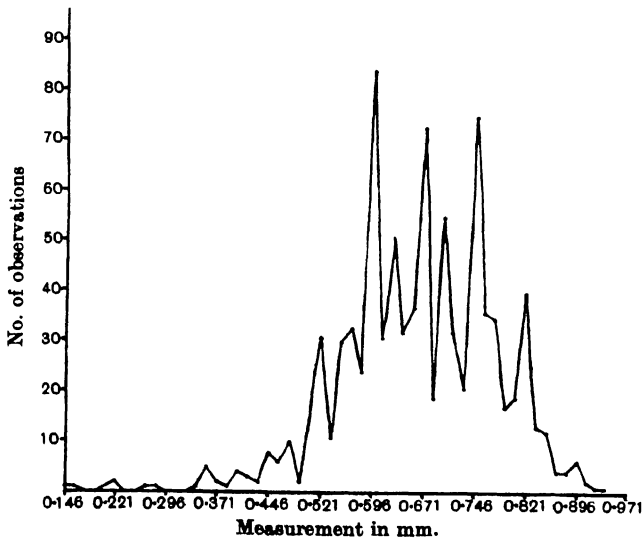
The width of the head capsule is seen to vary in size between the limits of 0.146 and 0.940 mm. with intervals of approximately 0.015 mm. Of the 887 head capsules 777 (87.59 per cent.) are found to measure from 0.521 to 0.821 mm. inclusive, 67 (7.55 per cent.) below 0.521 mm., and 43 (4.84 per cent.) above 0.821 mm.

Ignoring the measurements below 0.521 and above 0.821 mm. the graph shows nine distinct peaks with their corresponding troughs. These peaks appear to resolve themselves into two groups, a higher series at

0.521, 0.596, 0.671, 0.746 and 0.821 mm., alternating with a lower series at 0.566, 0.626, 0.701 and 0.776 mm. It is very evident from these figures that the explanation to be sought is no simple one.

Table I.

Measurement in mm.	No. of observations	Measurement in mm.	No. of observations
0.149	1	0.612	31
0.164	1	0.627	51
0.209	1	0.642	32
0.224	2	0.657	37
0.269	1	0.671	73
0.283	1	0.686	19
0.343	1	0.701	55
0.358	5	0.716	32
0.373	2	0.731	21
0.389	1	0.746	75
0.403	4	0.761	36
0.418	3	0.776	35
0.433	2	0.791	17
0.447	8	0.806	19
0.463	6	0.821	40
0.478	10	0.836	13
0.493	2	0.851	12
0.507	16	0.866	4
0.522	31	0.881	4
0.537	11	0.895	6
0.552	30	0.910	2
0.567	33	0.925	1
0.582	24	0.940	1
0.597	84		



Text-fig. 1.

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Three interesting points arise for discussion:

- (1) The absence of evidence suggesting well-defined instars.
- (2) The main irregularities of the graph, that is to say the small numbers of larvae with head capsules below 0.521 and above 0.821 mm.
- (3) The occurrence of the nine peaks between 0.521 and 0.821 mm.

### (1) *The absence of evidence suggesting well-defined instars.*

As has already been noted, the measurements of the head capsules do not fall into well-defined groups which may be taken to indicate the modes of given instars. Almost every size from 0.146 to 0.940 mm. with intervals of 0.015 mm. occurs. This can be taken as evidence that the head capsule is not of a constant size in any one instar, but that it varies within limits. It is also probable that the limits of instars overlap, that is to say that the largest individuals of a given instar may be larger than the smallest individuals of the next. According to Miles, this overlapping of instars also occurs in certain of the Tenthredinidae.

### (2) *The main irregularities of the graph.*

These may be readily accounted for in the following manner:

(a) *Periodicity in egg-laying.* The crest of egg-laying having occurred earlier in the year, the majority of the larvae had reached the stages measuring from 0.521 to 0.821 mm. The few larvae with head capsules below the 0.521 mm. may be very late individuals of the same brood, or the forerunners of the next brood.

(b) *Incomplete sampling.* The smallest larvae are exceedingly difficult to pick out from the cattle cake, even with the aid of a hand lens. It is therefore possible that a certain number of these were overlooked. It is considered, however, that this is not the case, but rather that the explanation set forward in (a) is a more correct statement of fact.

According to Munro (1915), the first stage larva in *Sitodrepa panicea* is campodeiform and not eruciform. Even the smallest of larvae examined for this study were eruciform, but it is possible that they had already undergone ecdysis.

(c) *Pupation.* Above the 0.821 mm. size very few larvae were found. This again may have been due to the fact that the largest number of the population had not yet reached this stage of development, i.e. due to periodicity in egg-laying. On the other hand it is likely that pupation supervenes at this stage, and that the few examples (4.84 per cent.) above the 0.821 mm. line are above the average in size.

(3) *The nine peaks between 0.521 and 0.821 mm.*

The occurrence of these nine peaks presents the most interesting problem. It is considered unlikely that the peaks should represent the modes of separate instars, since this would give a minimum of nine, a number hitherto unprecedented in the Coleoptera. Some other explanation must therefore be sought.

If the series of higher peaks (0.521, 0.596, 0.671, 0.746 and 0.821 mm.) be examined, it is seen to approximate roughly to a geometric progression with a common ratio of 1.12. This is shown in Table II.

Table II.

Actual mode mm.	Calculated mode mm.	Common ratio
0.521	—	1.12
0.596	0.584	"
0.671	0.654	"
0.746	0.733	"
0.821	0.821	"

In a similar manner the second series (0.566, 0.626, 0.701 and 0.776 mm.) approximates to a geometric progression having a common ratio of 1.11 (see Table III). From this it would appear as if two separate series of instars are under observation, the one series having modes at 0.521, 0.596, 0.671, 0.746 and 0.821 mm., and the other series at 0.566, 0.626, 0.701 and 0.776 mm.

Table III.

Actual mode mm.	Calculated mode mm.	Common ratio
0.566	—	1.11
0.626	0.629	"
0.701	0.699	"
0.776	0.776	"

It is therefore suggested that the two series represent the two sexes of the beetle. This would give a minimum of four instars for one sex, and five for the other. It is also possible that this difference in the number of instars is a constant feature, since Miles (*loc. cit.*) states that in the Tenthredinidae female larvae pass through six larval instars while the male only have five. Thus while not yet distinguishable by secondary sexual characters it appears to be a reasonable suggestion that male and female beetles are already differentiated in the larval stage by size. It is already known that male and female prepupae as well as pupae can be distinguished by their size, the female usually being the larger.

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Furthermore, if this hypothesis is correct, it would appear that the sex ratio is not unity, since the summation of the second series of peaks is smaller in number than that of the first series.

### III. SUMMARY AND CONCLUSIONS.

1. A number (887) of larvae of *Sitodrepa panicea* L. were examined with a view to testing the application of Dyar's law and the possibility of estimating the number of larval instars by measuring the head capsule of a random population.

2. The measurements were found to fall into two groups, one slightly larger than the other, whose growth ratios approximated to two geometrical series, one with a common ratio of 1.12, the other with a common ratio of 1.11.

3. It is suggested that these two groups represent the sexes.

4. On this hypothesis it would appear that the males undergo a minimum of four larval ecdyses, and the females a minimum of five. Miles (*loc. cit.*) postulates a similar phenomenon for several species of Tenthredinidae. Furthermore, the sex ratio is not unity.

5. The method of investigating the growth ratio of the larva by the measurement of a large population, is not entirely satisfactory. Owing to a diversity of causes such as the periodicity of egg-laying, insufficient numbers of the young larvae were obtained, and no satisfactory conclusions with regard to the number of these early instars can be reached.

6. The analysis of the data is further complicated by the variation in size and presumed overlapping of the instars in both sexes.

7. Until much larger and more representative populations, or many more samples of small populations can be examined, such a method of determining the number of larval instars in a given insect can be but of debatable value.

### IV. ACKNOWLEDGMENTS.

The work was carried out in the Department of Zoology, University College of Wales, Aberystwyth, under the supervision of Prof. R. D. Laurie, M.A., and Mr J. R. W. Jenkins, M.Sc., to whom the writer wishes to tender her thanks for advice and criticism. The writer also desires to record her gratitude to Dr H. F. Barnes and Dr R. A. Fisher of Rothamsted Experimental Station for their valuable help in the rewriting of this paper.

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(Received January 27th, 1932.)



# ON THE BIOLOGY OF THE APPLE SAWFLY, *HOPLOCAMPA TESTUDINEA* KLUG.

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(With Plates XXIV—XXVI.)

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## INTRODUCTION.

THE broad outlines of the life history in this country of the apple sawfly, *Hoplocampa testudinea* Klug., have been described by Westwood (10), Ormerod (5) and Theobald (9). The adults appear in the spring at the time of the blossoming of the apple and may be found about the blossoms, particularly in bright sunshine. The eggs are laid about the flowers and give rise to larvae a week to a fortnight later. The larvae tunnel into the fruitlets and eat out a large cavity in the centre, destroying the developing seeds. Should one apple be insufficient to satisfy their food requirements, a second fruit may be attacked. In from 4 to 6 weeks the larvae become fully fed and desert the fruit. They tunnel into the soil and spin cocoons in which they hibernate. There is normally only one brood per annum, and the insect remains in the cocoon as a quiescent larva from June to the following April or May.

In order to obtain further information regarding the habits of the adults, oviposition, larval habits and pupation, the species has been kept under observation in Lancashire and Cheshire since 1928. In this paper the accumulated data are recorded. Most of the important details were observed both in the field and in the laboratory, but since fruit failed to develop in the laboratory the data on larval growth were obtained from larvae collected in the field.

## DESCRIPTION AND HABITS OF THE ADULTS.

Morice (4), in his synoptic table of the British *Hoplocampa* spp., gives the following diagnostic characters of *H. testudinea*. Clypeus emarginate; head entirely black above; pronotum pale. Stigma black at base, pale at apex. Body pale fulvous brown, with vertex and the dorsal surface of the thorax and abdomen for the most part black and shining. Morice gives the size as 6-7 mm., and this is correct for specimens taken and bred by the present writer.

Mature larvae collected in July, 1928, over-wintered in the soil of a large breeding cage. Adults began to emerge indoors on April 29th, 1929, and were placed on small pot apple trees and kept in the laboratory for further observations. Observations made in the laboratory were subsequently verified in the field, and the following account of the habits of the adults is drawn from data thus obtained.

The adults are active, especially during the morning when the temperature is rising. In bright sunlight they fly rather unsteadily about the blossoming fruit trees, often settling on leaves or branches and running about with antennae quickly vibrating. During these periods of activity drops of moisture on the leaves were taken up and the surfaces of the leaves licked or scraped from time to time with the trophi. The flowers were entered occasionally and liquid which had collected at their bases was taken up. The period of maximum activity usually occurred about midday, activity gradually waning during the afternoon. When both sexes were present feeding, mating and oviposition could be observed during the periods of greatest activity.

When the sexes were confined together in the laboratory and kept under careful observation, it was found that there was no evidence of sexual excitement until the female was 4 days old. Then about an hour before midday, in bright sunshine, the males ran rapidly up the trunk, dropped to the soil and ran round waving their antennae continually and occasionally exerting the genitalia. This was repeated several times while the female rested more or less motionless on the soil beneath the tree. Gradually she became active, running for short distances with rapidly vibrating antennae. When in close proximity to the female the male became exceedingly excited, running up on her back and beating her head with his antennae, and at the same time exerting and retracting the genitalia. He then ran alongside the female with genitalia exerted and curled beneath the abdomen. At this time both insects faced in the same direction and were either walking or stationary. Interlocking then

took place and the male gradually swung round and faced in the opposite direction. Coitus continued for about 5 min., during which period the insects remained stationary. They then separated and remained rather inactive for some time, cleaning limbs, wings and antennae. A second male tried to mate with the same female from time to time but did not succeed until 6 days later, since on the approach of this male the female became restless and avoided him.

The following notes were taken in the fruit plantation at Reaseheath at a time when sawfly activity was exceptionally high. "11. v. 31. Both sexes of *H. testudinea* numerous and active on apple blossom of variety 'Bismarck.' Many in open flowers eating pollen and thrusting their heads into nectar and into moisture at the base of the style. Many pairs mating, some in copula for upwards of a minute, others for less than three-quarters of a minute. Many females active amongst the flower stems of the trusses and quite hidden by the open petals. Some females ovipositing. Sometimes a female under observation oviposited in several flowers of a truss, at other times only in one or two, the latter usually when the sunlight was very intense."

Observations out of doors indicated that mating took place about the trees and not on the soil as had occurred in the laboratory. Mating on the soil in the laboratory seemed to result from the adults being confined on seedling trees which were not large enough to permit the normal activities of the insects.

During oviposition the female usually takes a position with the head directed towards the base of the flower truss and the ovipositor in contact with the receptacle just below one of the sepals. The body is then arched and the saws, which are held vertically, penetrate the receptacle. They are pushed inwards and upwards, and then directed towards the right of the insect where they are turned on to a more or less horizontal plane. A pocket-like cavity is then cut on this horizontal plane immediately beneath the epidermis of the upper surface of the receptacle, within the ring formed by the bases of the stamens.

Two other positions were occasionally assumed by females during oviposition. Sometimes the insect rested in the cleft between two sepals, facing the interior of the flower and curving the tip of the abdomen downwards on to the receptacle. The ovipositing insect was also observed to rest on the inner surface of the open petals with the head directed towards the outer edge and with the abdomen directed into the flower so that the ovipositor entered the tissue of the receptacle between the styles and the stamens. In both these positions the insect deposited the

egg in a position somewhat similar to that obtaining when the usual position associated with oviposition was assumed.

The time required for the operation of oviposition varied between 1 and  $1\frac{1}{2}$  min., and most usually occupied  $1\frac{1}{4}$  min. While females were ovipositing males were active about the blossom trusses and often disturbed the females in the act of oviposition. At such times the females either desisted or the operation was performed imperfectly. Occasionally the egg was pushed through the epidermis and left lying wholly or partially exposed upon the upper surface of the receptacle within the circle of stamens. Though insect activity fluctuated in direct relation to the wind and sunlight and was generally greatest about midday, females were occasionally observed ovipositing in the late afternoon when there was bright sunshine and still, warm conditions.

In the laboratory adults lived 6-11 days, though 6-9 was the usual period. Adults collected in the open on May 11th were sleeved on branches of apple trees and kept under observation. The last of these insects, a female, died on May 22nd, and the period of adult life under these conditions seemed to be similar to that observed in the laboratory, though the date of emergence of these insects was unknown.

#### THE EGG STAGE.

After the ovipositor of the female has been withdrawn from the receptacle there is usually a slight exudation from the point of insertion of the saws. This exudation causes a brown discoloration on the pubescence of the receptacle and affords a fairly reliable means of detecting infested blossoms. Fig. 3 shows an oviposition scar 3 days old in a typical position at the base of a sepal.

The egg of the apple sawfly can generally be seen, as in Fig. 4, in the infested blossom if the stamens are removed and the upper surface of the receptacle, between the calyx and the styles, is examined. It becomes markedly apparent a few days after it is laid, for, as is characteristic of many sawfly eggs<sup>(6)</sup>, it increases slightly in size after it is laid, and this swelling almost invariably causes the epidermis of the receptacle to rupture, leaving the egg partly exposed. Fig. 5 shows the egg in the usual position in the receptacle: a sepal has been removed and the egg is seen to lie beneath the bases of two stamens but projecting beyond them.

The shape of the egg of *H. testudinea* is shown in Fig. 5. It is about 0.8 mm. in length, slightly curved, rounded at the cephalic end and tapering slightly towards the distal end. In almost every case where developing eggs were kept under observation, they became partly exposed

during incubation owing to the rupturing of the epidermal tissue of the receptacle. Eggs laid on May 11th hatched on May 26th after an incubation period of 15 days; some eggs laid on May 18th also hatched on May 26th after an incubation period of 8 days, while others laid on May 18th hatched on May 30th after an incubation period of 12 days. These observations suggest that there may be considerable variation in the incubation period, and under the climatic conditions prevailing in Cheshire in May, 1931, the observed incubation period was 8–15 days.

In 1932 the incubation period was even longer, being 16–18 days in observed instances.

#### THE LARVAL STAGE.

For some hours before eclosion the larva can be seen moving its jaws more or less vigorously in an endeavour to rupture the chorion. When this is achieved the larva draws itself out from the egg. Since the overlying epidermis of the receptacle is usually ruptured during incubation and the cephalic end of the egg exposed (Fig. 4), the larva escapes on to the receptacle within the circle of sepals.

The young larva then endeavours to enter the fruit. Occasionally<sup>1</sup> it enters from within the calyx ring and makes a tunnel penetrating directly to the ovary. Fruitlets attacked in this manner appear normal, as there is no sign of infestation outside the calyx ring. As a rule, however, the larva leaves the calyx cup and tunnels between two sepals or enters the fruit just below the calyx ring. Under these circumstances a long winding tunnel, which may or may not reach the ovary, is often made. If the ovary is ultimately reached only one or two, seldom more, of the seeds are attacked, and these are not always entirely devoured.

At the time of attack the young fruits are expanding rapidly with the result that the epidermis over the tunnels, which frequently lie immediately below the surface, splits along the path of the tunnel, and tiny winding brown scars containing frass become apparent on the fruitlets (Fig. 6). Examination of fruitlets showing these scars revealed that in many instances the larva had failed to reach the ovary or having done so had died without feeding upon it. In such circumstances the fruit continued to develop, but when mature bore the ribbon scar (Fig. 7) described by Petherbridge (7).

When the larva penetrates to the ovary and feeds upon the developing seeds the fruit usually falls to the ground, but before this occurs, about a

<sup>1</sup> In two examples out of 100 fruits of "James Grieve" examined in June 1932 entrance was effected within the calyx ring.

fortnight after its entrance and often before the seeds are exhausted, the larva migrates to another fruit. The second fruit is usually much larger than the first owing to its having escaped attack during the earliest stages of development. The larva, which has by this time reached the third instar, tunnels directly to the ovary so that there are no winding scars associated with this phase of sawfly attack. The fruitlets attacked by first instar larvae seldom contain more than one insect, but fruit entered by migrating larvae may contain two or three insects. Moreover, fruitlets invaded by newly emerged larvae are small, stunted or malformed, dark green and somewhat pubescent owing to interrupted development in the initial stages of growth, while fruits invaded by migrating larvae are much larger, brighter green, without pubescence and showing a circular entrance hole of about 1.5 mm. in diameter, usually in the stem half of the apple. Fig. 8 shows the entrance holes of migrating larvae.

On entering the second apple the larvae feed rapidly and voraciously and destroy not only the seeds but frequently the ovary walls and some of the tissue of the receptacle. Where two or three migrating larvae enter the same fruit the interior is soon devoured and the larvae may migrate to a third fruit. When much of the attacked fruit falls to the ground the larvae may be found feeding in the fallen apples. If not mature they may migrate to other fruit and can be found crawling up the trunks of the trees in search of new feeding sites. This was observed at Reaseheath in 1929 and 1931.

At eclosion the larvae of the apple sawfly measure 1.75 mm. when fully extended. In the early instars they are a whitish colour, with the head shining black at the vertex but paler on the facial area. Black or dark brown chitinous plates occur on the dorsum of the eighth, ninth and tenth abdominal segments. Those on the ninth and tenth are conspicuous and cover almost the entire dorsal surface, while on the eighth segment there are two narrow brownish plates following the lines of the annulets. Pale, microscopic setae occur on the head and on the caudal sclerites and also on the legs, prolegs and entire body. The thoracic legs are well developed, pale but darkening towards the edges of the sclerites and with a distinct brown tarsal claw. Prolegs occur on segments 2, 3, 4, 5, 6, 7 and 10 of the abdomen, those of the tenth segment being considerably enlarged. The spiracles are small and elongate, and are present on the prothorax and the first eight abdominal segments (Fig. 9).

At the fifth instar the larvae become mature, and measure 9–11 mm. Their general body colour is whitish and the annulation of the segments

is pronounced. In contrast with the preceding instars, in the fifth instar the head is yellowish brown with the eyes and mandibles brown. The thorax is broader and stouter than the abdomen which tapers rather sharply caudally. The dorsal plates of the caudal segments, which were conspicuous in the preceding instars, are pale with faint darker pits and short pale setae and are scarcely visible on the whitish body.

Injured fruits were collected at weekly intervals from the Reaseheath fruit plantation during 1931, and examined to obtain data on the intensity of attack and the growth of the larvae. Table I summarises the data collected.

Table I.

Date	No. of injured fruits	No. of infested fruits	No. of larvae present in each fruit			Total no. of larvae	Instar of the larvae				
			1	2	3		1st	2nd	3rd	4th	5th
30. v.	24	17	16	1	—	18	16	2	—	—	—
3. vi.	25	21	21	—	—	21	3	18	—	—	—
10. vi.	41	37	36	1	—	38	—	3	35	—	—
18. vi.	40	34	31	3	—	37	—	—	2	35	—
23. vi.	44	26	21	4	4	41	—	—	2	9	30

Observations in the field indicated that oviposition usually occurs within a few days of the opening of the blossom of the earliest varieties in the plantation, and the larvae reach maturity 6–7 weeks later. Table I shows that larval development is fairly regular and rapid. By June 23rd, 1931, almost exactly 6 weeks after the commencement of oviposition, 75 per cent. of the larvae had reached the fifth instar. Though there seems some variation in the period of larval growth from year to year, the following observations indicate that it is only slight. In 1929 the first larvae collected from Reaseheath matured and entered the soil on June 25th; in 1930 larvae from the same plantation entered the soil on June 26th, and in 1931 on June 24th.

Table II.

Instar	Head widths in millimetres	Average	Growth ratio
1st	0.34, 0.38, 0.38, 0.38, 0.38, 0.38, 0.42, 0.42, 0.42	0.392	—
2nd	0.53, 0.53, 0.53, 0.53, 0.53, 0.57, 0.57, 0.57, 0.57	0.550	1.4
3rd	0.76, 0.76, 0.78, 0.80, 0.80, 0.80, 0.82, 0.82, 0.82	0.786	1.4
4th	1.07, 1.07, 1.08, 1.10, 1.10, 1.10, 1.12, 1.12, 1.14, 1.16	1.106	1.4
5th	1.49, 1.49, 1.53, 1.53, 1.53, 1.53, 1.57, 1.57	1.526	1.38

Observations were made on the width of the head capsule in the larval instars to obtain information on the rate of growth and to afford a check for the observations given in Table I. The measurements fell into five well-marked groups, indicating five larval instars. The growth ratio at ecdysis was fairly constant during larval life and closely approximated that observed previously in other species (3). Table II gives details of measured head widths.

#### HIBERNATION AND PUPATION.

The mature larvae of *H. testudinea* enter the soil and construct cocoons in which they remain until the following spring. Theobald (9) states that the cocoons are 1-4 in. from the surface, but Masee (2) mentions the finding of cocoons at considerable depths in the soil.

In order to obtain data on the depth at which pupation takes place, a glass cylinder 10 in. deep was filled with soil made fairly compact to resemble the condition of plantation soil, and encased in folds of thick paper to shut out the light. Thirty larvae were placed on the soil and the top screened with gauze. In November the soil was removed in 3-in. layers and examined. A total of twenty-five cocoons were recovered. These were distributed in the soil as follows: three in the surface 3 in. of soil; eight at a depth of 3-6 in.; eleven at a depth of 6-9 in., and three more than 9 in. deep. From these figures it would appear that 75 per cent. of the cocoons were constructed in the soil at a depth of 3-9 in. and the remaining 25 per cent. occurred above and below this depth.

Fig. 10 shows that the parchment-like cocoons are regular in shape. They are difficult to find because they assume the colour of the soil in which they are found, and this probably explains the lack of accurate data regarding the usual depth of cocooning. There is some variation in the size of the cocoons, the length of those illustrated being 7.25-8.25 mm. and the width 4-4.25 mm., and the usual size 8 by 4 mm.

Within the cocoon the larva passes into the contracted prepupal condition without further ecdysis and retains this form until pupation. A few days before pupation the compound eyes and later the ocelli can be seen through the larval integument. The pupal ecdysis occurs about 3-4 weeks prior to emergence, the pupal period being 26-30 days. Sawfly pupae are remarkably constant in form, but there are frequently colour differences which become more pronounced as development proceeds. Shortly before emergence the pupae of *H. testudinea* (Fig. 11) assume a brownish tint which gradually darkens as the time for emergence approaches. They are active and rotate continually within



their cocoons. After emergence from the pupal skin the imago remains within the cocoon until the integument hardens and the typical reddish coloration is assumed. At emergence the insect cuts off a roughly circular cap from the end of the cocoon.

VARIETAL SUSCEPTIBILITY AND INFESTATION  
IN RELATION TO BLOSSOMING.

In 1931 data was collected at Reaseheath on infestation by larvae of *H. testudinea* of the different varieties in the plantation. Table III gives particulars for fifteen varieties, arranged as far as possible in the order of blossoming.

Table III.

Variety	Date of flowering	No. of fruits examined	No. of fruits infested	% infested	
Bismarck	May 11th	413	60	14.5	Average % infestation for early period 12.9
Beauty of Bath	" 12th	203	59	29.0	
Early Victoria	" 14th	494	16	3.2	
Grenadier	" 14th	413	20	4.8	
Stirling Castle	" 15th	267	44	16.4	Average % infestation for middle period 25.3
Lane's Prince Albert	" 15th	401	72	17.9	
Bramley Seedling	" 15th	263	33	12.5	
Rival	" 16th	376	68	18.0	
James Grieve	" 17th	233	94	40.3	Average % infestation for late period 9.1
Worcester Pearmain	" 18th	195	87	44.6	
Cox's Orange Pippin	" 19th	348	27	7.7	
Allington Pippin	" 19th	462	36	7.7	
Newton Wonder	" 19th	233	50	21.4	
Lord Derby	" 20th	321	22	6.8	
Lady Sudeley	" 21th	249	5	2.0	

From this table it is seen that though both early and late blossoming varieties may be heavily infested, those varieties flowering during the mid-blossoming period are most uniformly heavily infested. Varietal susceptibility, however, seems more important than date of blossoming, and scent, abundance of pollen and nectar may determine the degree of attraction of particular varieties for the insects, and so give rise to heavy infestations such as occurred on "James Grieve" and "Worcester Pearmain," and cause such differences in intensity of infestation as that noted on "Bismarck" and "Beauty of Bath," both of which were blossoming profusely on May 12th.

Data concerning infestation intensity was collected on June 26th, when the second phase of sawfly activity was in progress. The figures do not, therefore, indicate the total number of fruits destroyed by the larvae. There is normally some fall of fruit shortly after setting, and the coincidence of this fall with that resulting from the first phase of sawfly injury renders it difficult to assess the full extent of sawfly injury. Petherbridge

and Tunnington<sup>(8)</sup>, recording apple sawfly injury to "Worcester Pearmain" apples under observation in Huntingdon, estimated that 85-90 per cent. of the fruit was injured about a month after the last petals had fallen. In 1931 at Reaseheath the extent of the injury to "Worcester Pearmain" fruits was estimated at 45 per cent. 4-5 weeks after the petals had fallen.

#### CONTROL MEASURES IN RELATION TO BIOLOGY.

Lead arsenate and soap and nicotine sprays appear to have been used most successfully for the control of apple sawfly. Grub and Bagenal<sup>(1)</sup> record that lead arsenate applied before blossoming had no effect on the amount of injury by apple sawfly, but applied after blossoming resulted in a considerable reduction in the number of infested fruits. In 1926 Petherbridge and Tunnington<sup>(8)</sup> used arsenate of lead at the rate of 6 lb. per 100 gallons of water on fruit trees of the variety "Worcester Pearmain." The spray was applied before the petals had fallen, and no appreciable difference in the amount of injury could be observed on the sprayed and unsprayed plots, practically the entire crop being lost as a result of sawfly attack. In 1927 and 1928 these workers used a spray consisting of 4 oz. nicotine (95-98 per cent.), 4 lb. soft soap and 40 gallons of water. The spray was applied 7 days after the petals had fallen, and in 1927 the weight of marketable fruit was increased on the sprayed plots by 200 per cent., and in 1928 by 700 per cent.

From a study of the biology of the apple sawfly it would appear that control measures must be directed against the insect before it is able to penetrate the fruit. Since the egg is usually partly exposed on the upper surface of the receptacle during the later stages of incubation and the larva escapes from the egg on to the receptacle, it seems apparent that the spray must be timed and applied so that it reaches the surface of the fruit within the calyx ring. The failure of Grub and Bagenal to obtain control of the pest by the use of lead arsenate spray prior to blossoming was caused by the spray not reaching the portion of the receptacle attacked by the larvae. The reason for the failure of Petherbridge and Tunnington to obtain control of the pest with lead arsenate is less obvious. They record that the spray was applied before the last petals had fallen. It seems possible that the fruitlets still carried so much of the remains of the flowers that the spray did not penetrate within the calyx ring. The use of soap and nicotine sprays 7 days after the petals had fallen seems to have been successful because the drenching sprays could penetrate into the calyx cup and reach exposed eggs and emerging larvae.

It seems apparent that the time of application will be the critical factor in regard to the control of apple sawfly, and that sprays should be applied before rather than after the hatching of the larvae. It seems essential that the spray should not be applied until after the petals have fallen in order to ensure that the spray fluid penetrates within the calyx cup. It is probable also that the relation of the sepals to the calyx cup and the filaments to the surface of the receptacle after the fall of the petals may be of significance, for the temporary closing of the sepals after flowering and the drawing inwards of the filaments would afford some measure of protection to sawfly eggs and hatching larvae.

#### SUMMARY.

The biology of the apple sawfly, *Hoplocampa testudinea* Klug., has been under observation for several years in the north-west of England.

Mating and oviposition are described and an account is given of the position of the eggs on the host plant. Usually the eggs are found under the epidermis of the upper surface of the receptacle within the calyx ring, and often within the bases of the stamens.

The incubation period normally varied from 8 to 15 days. During this period the eggs increased slightly in size, and the majority ruptured the epidermis of the receptacle and so became partly exposed within the calyx ring.

The larvae emerge from the eggs on the outside of the fruitlet and proceed to penetrate it either from within the calyx ring, or by passing over the calyx on to the side of the receptacle and tunnelling in from that position. It appears that the larvae seek the ovary but numbers never reach it, and at this stage there may be some mortality among the larvae. Larvae which reach the ovary feed for a short time on the developing seeds and at about the third instar migrate to older fruits. Circular or ribbon scars appear to be caused by the wanderings of first instar larvae in their first penetration of the fruit.

The larvae are described. They have five instars and a growth ratio of about 1.4. They measure 9–11 mm. when mature.

The larvae over-winter in cocoons, the majority of those under observation were constructed at a depth of 3–9 in. in the soil. The pupal period lasts 3–4 weeks.

Notes are given on infestation by apple sawfly in relation to variety and date of blossoming of a number of apple varieties, and the significance of details of the biology of the species on control measures is discussed.

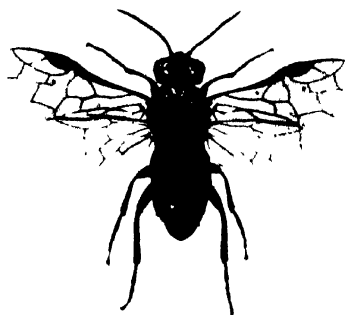


Fig. 1.

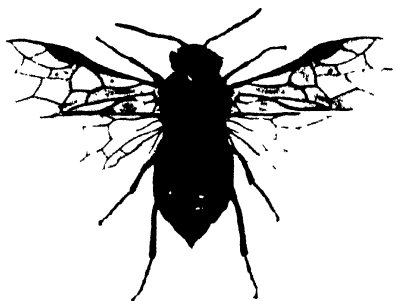


Fig. 2.



Fig. 3.



Fig. 4.





Fig. 5.



Fig. 6.



Fig. 7.



Fig. 8.





Fig 9

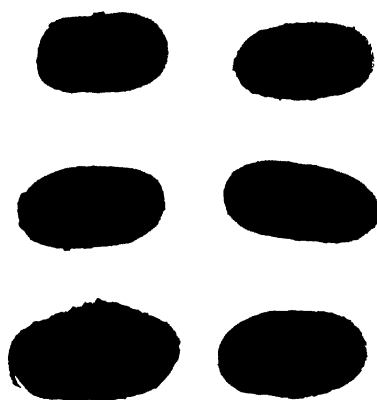


Fig 10



Fig 11.





It is suggested that the time of application of sprays is a critical factor in securing control of apple sawfly.

Acknowledgments are due to Mr W. B. Mercer, M.C., B.Sc., and Mr W. E. Shewell Cooper for facilities for observation at Reaseheath, Cheshire, and to Mr J. J. Green and Mr N. J. Macpherson for facilities for observation at Hutton, Lancashire. Thanks are also due to Mrs Mary Miles, M.Sc., for assistance during the course of the work, and to Mr F. R. Petherbridge, M.A., for criticism of the MS.

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### EXPLANATION OF PLATES XXIV—XXVI.

#### PLATE XXIV.

- Fig. 1. *H. testudinea*, male.  $\times 5$ .
- Fig. 2. *H. testudinea*, female.  $\times 5$ .
- Fig. 3. Site of oviposition of *H. testudinea*, scar 3 days old.  $\times 3$ .
- Fig. 4. Apple flower with petals and stamens removed to show egg of *H. testudinea* protruding on to surface of receptacle.  $\times 3$ .

#### PLATE XXV.

- Fig. 5. Apple flower with egg of *H. testudinea* in position at base of stamens near the upper surface of the receptacle.  $\times 5$ .
- Fig. 6. Apple fruitlets with winding tunnels characteristic of the first invasion by larvae of *H. testudinea*. The epidermis has ruptured with growth and the tunnels are exposed.  $\times 3$ .
- Fig. 7. Apples at maturity showing ribbon scars the result of early infestation as in Fig. 6. Natural size.
- Fig. 8. Young apples showing entrance holes made by fourth and fifth instar larvae of *H. testudinea*. Natural size.

#### PLATE XXVI.

- Fig. 9. Larvae of *H. testudinea*. The upper figures are fourth instar larvae with black heads and dark caudal plates the lower figures are fifth instar larvae with brown heads and very pale caudal plates.  $\times 5.5$ .
- Fig. 10. Cocoons of *H. testudinea*.  $\times 4$ .
- Fig. 11. Cocoon of *H. testudinea* opened to show pupa.  $\times 16$ .

(Received January 26th, 1932.)

# THE TOXICITY OF THE VAPOURS OF VOLATILE ORGANIC COMPOUNDS TO THE "RED SPIDER" MITE (*TETRANYCHUS TELARIUS* L.)

## PART I. SOME ALIPHATIC ALCOHOLS AND THEIR FORMIC ESTERS

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### INTRODUCTION.

MANY volatile organic compounds have been tested as fumigants against the "red spider" mite, *Tetranychus telarius* L., infesting glasshouse crops, but apparently there have not been previous investigations regarding the relation between the chemical constitution and the toxicity of the vapours of compounds towards this mite.

Whilst the insecticidal values of esters of several aliphatic alcohols have been determined in connection with the fumigation of stored products the alcohols have received little attention. Moore(8) found the toxicity of methyl, ethyl and amyl alcohols towards the housefly, *Musca domestica*, increased with increasing molecular weight, whilst Neifert and his co-workers(10) obtained similar results in experiments with grain weevils, with the exception that ethyl alcohol was less active than methyl alcohol. Holt(3) found methyl alcohol more toxic than ethyl or amyl alcohol to cockroaches.

The esters of aliphatic alcohols are of considerable insecticidal importance, since ethyl formate is used in fumigating certain food products, and patents covering the use of esters of formic acid have been granted in several countries. There is little information, however, as to the relative toxicities of the vapours of the homologous series of formates in relation to their constitution and chemical and physical properties. Cotton and Roark(2) determined the lowest concentrations of several alkyl formates necessary to kill rice weevils, *Sitophilus oryza* L., but their method, though resembling large-scale fumigating conditions, did not indicate the true relative toxicities of the compounds owing to differences in rates of penetration and adsorption by the mass of grain present.

## EXPERIMENTAL.

Glass bottles of about 3 litres capacity were fitted with rubber stoppers having inserted in them short lengths of glass tubing of sufficient size to admit the pipette and closed at the ends with rubber stoppers. Small hooks on the undersides of the large stoppers enabled the tube containing the mites to be suspended in the bottles by a length of cotton. A small piece of filter paper was placed at the bottom of each bottle in such a position that it could be touched with the tip of the pipette, which was made from a length of capillary tubing (0.5 mm. bore) marked off in half centimetres and long enough to reach the bottom of the bottle when inserted through the tube in the stopper. This pipette was calibrated for each substance tested.

The mites were placed in lengths of glass tubing about 3 cm. long and 2 cm. in diameter, closed at either end with pieces of very thin silk fabric held in position with thin rubber bands. Owing to difficulties in transferring the mites to and from the tubes undamaged on account of their minuteness and the manner in which they frequently formed webs when irritated by vapours, it was found necessary to insert them on pieces of tomato foliage about 2 square cm. in area. By careful selection it was possible to include well over forty adult mites in each tube. The "resting" stages of the mites were removed from each test-piece.

The bottles were placed in a thermostatic chamber (25° C.), and when they had attained this temperature the compound was introduced by inserting the pipette, after wiping its exterior, through the glass tube and allowing the desired amount to run out. Before withdrawing the pipette the tip was brought into contact with the piece of filter paper to remove any liquid adhering to the end. The stopper was inserted in the tube immediately after withdrawing the pipette, and the bottle kept in the chamber for 5 hours. It was found that the volatilisation of the least volatile substances tested was complete in less than 15 min., and although in some of the earlier tests the contents were mixed by inverting the bottles several times this was found unnecessary, presumably owing to the convection currents produced as a result of heating the chamber from below.

After fumigation the pieces of leaf carrying the mites were placed in the open for several hours and then placed on fresh tomato leaflets in glass-topped boxes, the whole being kept moist by the inclusion of pieces of damp cotton-wool. The mites were examined daily for 4 days, and if any mites showed movement at the end of this period it was taken

that a 100 per cent. kill had not been obtained. The minimum concentration of each substance necessary to kill 100 per cent. of adult mites under these conditions was determined several times for each substance.

### RESULTS.

The minimum amount required to kill 100 per cent. of the mites under the prescribed conditions expressed in grams per 1000 litres is termed the lethal concentration of the substance, whilst the molecular weight of the substance divided by the lethal concentration is termed the molecular toxicity and is of greater value for comparative purposes than the lethal concentration. The values obtained for a number of aliphatic alcohols and their formates are shown in Table I.

Table I.

*Lethal concentrations and molecular toxicities of the vapours of aliphatic alcohols and their formates to Tetranychus telarius L.*

	Alcohol		Formate	
	Lethal conc.	Molec. toxicity	Lethal conc.	Molec. toxicity
Methyl	54.0	0.6	3.2	19.0
Ethyl	46.0	1.0	9.8	7.5
<i>n</i> -Propyl	28.0	2.1	7.1	12.4
<i>iso</i> -Propyl	33.0	1.8	9.6	9.2
<i>n</i> -Butyl	12.6	5.9	6.2	16.5
<i>iso</i> -Butyl	14.2	5.2	6.9	14.8
<i>sec.</i> -Butyl	17.5	4.2	8.6	11.8
<i>tert.</i> -Butyl	39.5	1.9	—	—
<i>n</i> -Amyl ( <i>n</i> -Butyl carbinol)	7.2	12.3	7.9	14.7
<i>iso</i> -Amyl ( <i>iso</i> -Butyl carbinol)	7.2	12.3	8.3	14.0
<i>act.</i> -Amyl ( <i>sec.</i> -Butyl carbinol)	9.5	9.2	11.4	10.0
<i>sec.</i> -Amyl (methyl <i>n</i> -propyl carbinol)	11.2	7.9	12.3	9.2
<i>tert.</i> -Amyl (dimethyl ethyl carbinol)	15.8	5.6	21.0	5.5
Diethyl carbinol	13.8	6.4	15.0	7.7

Table I shows that the toxicity of the normal aliphatic alcohols increases as the series is ascended, whilst the toxicity of their formates appears to reach a maximum with *n*-butyl formate. Methyl formate is an outstanding exception to this generalisation. In addition it is seen that the normal compounds are more toxic than their isomers, the toxicity decreasing with increasing branching of the chain and this decrease being greatest when the branching is at the  $\alpha$  position to the hydroxyl or carboxyl group. There does not appear to be any appreciable difference in the toxicities of normal and *iso*-amyl alcohols although *n*-amyl formate was slightly more active than *iso*-amyl formate.

The relative toxicities of methyl and ethyl alcohols are of interest, since Holt(3) found methyl alcohol more toxic to cockroaches than ethyl

or amyl alcohols, and Neifert(10) states that methyl alcohol is more toxic than ethyl alcohol to grain weevils, but with this exception the toxicity of the vapours of aliphatic alcohols increased with increasing molecular weight. Morgan and Cooper(9), using *B. typhosus* and *Staphylococcus pyogenes aureus*, and Kamm(4) working with *Paramecia*, have shown that the toxicity of the normal alcohols increases as the homologous series is ascended with the exception of methyl alcohol, whilst Stadler(15), using *Staphylococcus aureus*, *Bacillus coli* and *B. pyocyaneus*, found methyl alcohol less toxic than ethyl alcohol. Uppal(17) also found that the toxicity of aliphatic alcohols in aqueous solution to the spores of *Phytophthora colocasiae* increased from methyl alcohol as the series was ascended, and that the normal alcohols were more active than their isomers. The greater physiological activity of the normal compounds as compared with their isomers has also been demonstrated by Morgan and Cooper(9), Vernon(18), Macht(7), Lendle(6) and Tilley and Schaffer(16).

The greater toxicities of the formates of the *iso* and *sec.* alcohols as compared with the normal formates found by Cotton and Roark(2) are possibly due mainly to the greater adsorption of the normal compounds by the wheat present in his experiments and partly to the greater volatility of the branched chain compounds.

#### *Toxicity and volatility.*

Although the toxicity of the substances tended to increase with increasing boiling-point there is no definite relation between the volatility of the compounds as expressed by their boiling-points and their toxicities to the mite. Neither is there any correlation between the toxicities of certain of the formates and their vapour pressures at the temperature at which the tests were made.

#### *The nature of insecticidal action.*

Without doubt the physical properties of members of a homologous series are of great importance in connection with their physiological activity. Although a large amount of information is available regarding the effect of physical properties upon the penetration of membranes, narcotic action, etc., the problem of insecticidal action especially in respect of the action of fumigants is too complex to allow of easy interpretation based on any one or more property. In addition the minuteness of *Tetranychus telarius* renders it impossible to determine which organs are affected by the vapours of the substances tested.

That the physiological activity of the substances is related to some

extent with their adsorbability seems probable in view of recent work on membrane penetration. Rideal (12) states that with regard to the permeability of membranes "the data support an adsorption rather than a solution mechanism," whilst Bayliss has also criticised the lipid-solubility theory of Overton (12).

Schilov and Nekrassov (14) have shown that the adsorption by charcoal of members of homologous series increases on the addition of  $-\text{CH}-$  groups until a maximum is reached, and in general compounds of normal structure are more strongly adsorbed than those of *iso* structure. Langmuir (5) and Adam (1) have found that the hydroxyl and carboxyl groups are polar to water and the hydrocarbon chains orientated away from the surface of the water. Should somewhat similar conditions prevail on the cell walls of the mite it is conceivable that the decreased toxicity due to the branching of the chain may primarily be due to a decreased adsorption on the cell surfaces.

Evidence with regard to the activity of the hydroxyl group in the alcohols is conflicting, but in general there does not appear to be any connection between the toxicity and hydroxyl activity of the alcohols as determined so far.

*The effect of the vapours of the aliphatic alcohols and their formic esters on tomato plants.*

Since these experiments have been made with the object of finding a fumigant which can be used in controlling the mite *Tetranychus telarius* on glasshouse plants (in particular on tomatoes) tests have been made to determine the tolerance of tomato plants to the vapours of these substances. For this purpose plants about a foot high and in 4 in. diameter pots were placed in the thermostatic chamber and subjected to 5 hours' exposure at 25° C. to the vapours resulting from a measured amount of the liquid poured on the floor of the chamber. After treatment the plants were removed to a glasshouse for observation.

The tests were conducted in the dark and, whilst the plants were all of the same variety and as far as possible of the same age and type of growth, it was found that the results obtained from individual plants under identical conditions varied considerably, and in consequence the values given are the mean values from several plants. It was found that the soil moisture had no appreciable influence on the damage caused to plants provided they were not wilting on account of dryness.

The results in Table II show the amounts of the substances which tomato plants are able to endure without injury under the conditions of

the test and are termed the "tolerances" of the substances. It will be seen that the effect of molecular structure is even more pronounced with regard to the action of homologues on plants than on the mites.

Table II.

*Tolerance of Tomato plants to the vapours of aliphatic alcohols and their formates.*

	Tolerance, g. per 1000 litres	
	Alcohol	Formate
Methyl	80	4
Ethyl	80	8
<i>n</i> -Propyl	28	5
<i>iso</i> -Propyl	40	15
<i>n</i> -Butyl	15	5
<i>iso</i> -Butyl	20	9
<i>sec.</i> -Butyl	26	10
<i>tert.</i> -Butyl	29	—
<i>n</i> -Amyl	8	5
<i>iso</i> -Amyl	10	5
<i>act.</i> -Amyl	15	7
<i>sec.</i> -Amyl	15	9
<i>tert.</i> -Amyl	27	23
Diethyl carbinol	18	18

Although in the majority of cases the amounts of the substances tolerated by the plants appear in excess of the concentrations lethal to the mites, it was found that in no case could the mites be destroyed under the conditions of these latter tests unless amounts in excess of those found lethal to the mites in the previous tests (Table I) were used. This is attributed to the loss of the vapours from the chamber by diffusion and to absorption by the soil, plants, etc.

#### SUMMARY.

1. With the exception of methyl formate the toxicity towards the "red spider" mite of the normal aliphatic alcohols and their formates at first increases as the series is ascended.
2. Methyl formate is an exception to the above rule and is the most toxic of the formates tested.
3. The normal compounds are more toxic than their isomers, the toxicity decreasing with increased branching of the chain.
4. The above decrease is greatest when the branching is at the  $\alpha$  position to the hydroxyl or carboxyl group.
5. The relative effects of the compounds on plants are similar to their effects on the mite, but the differences due to molecular structure are more pronounced.



6. Control of the mite cannot be obtained on tomato plants without injury by the use of any of the substances tested.

The author wishes to express his indebtedness to Dr W. F. Bewley and Mr E. R. Speyer for the interest they have shown in this work.

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(Received December 17th, 1931.)

## REVIEWS

*Sulphur Bacteria.* A monograph. By DAVID ELLIS. London and New York: Longmans, Green and Co. 21s. net.

The subject of autotrophic bacteria, or those organisms which are able to obtain their energy chemosynthetically, namely, by the oxidation of certain elements or their simple inorganic compounds, has been largely neglected by the botanist and the bacteriologist alike. Frequently they are relegated to certain special branches of bacteriology, such as soil bacteriology or water bacteriology, in view of the fact that these forms occur abundantly in the soil or in water, which seems to be sufficient excuse for the general bacteriologist to neglect completely the nature and functions of this abundant and important group of micro-organisms. Not only their wide distribution in nature, as in sea waters and in lake waters, in peat bogs and in various swampy regions, in soil and in sewage, but also their highly specific physiology, which places them apart from other groups of micro-organisms, makes their study highly attractive and instructive.

The appearance of a special monograph devoted entirely to a group of bacteria is a rare enough phenomenon, and it becomes especially so when this group is found to belong to the autotrophic bacteria. One would look, therefore, with considerable interest and expectation to the appearance of a monograph on sulphur bacteria, especially in the English language, since most of the monographic treatment of these organisms has been previously published largely in German. However, the monograph under consideration will not only not justify these expectations, but will leave one totally disappointed and discouraged. In spite of the classical investigations of Winogradsky, in spite of the contributions of Beijerinck, Omeliansky, Keil, Duggeli, Lieske, Trautwein, Baas-Becking, van Niel, and others, on this group of organisms, especially their physiological activities and the rôle of sulphur in their life cycle, the author has completely misunderstood their specific physiology. On the one hand, a misconception is introduced by suggesting that the term "sulphur bacteria" should be applied only to those organisms which attack hydrogen sulphide, but not to the true sulphur-oxidising bacteria, thus leaving out practically all the sulphur bacteria belonging to the Eubacteriales from consideration; on the other hand, the sulphate-reducing bacteria and the saprophytic bacteria which liberate hydrogen sulphide in the decomposition of proteins, are placed on a par with the true sulphur-oxidising bacteria. If an accurate knowledge of the physiology of an organism is essential for an understanding of its relation to other related forms in the case of bacteria in general, it certainly holds true for the various groups of autotrophic bacteria. However, the author almost overlooks the specific physiology of these organisms, if one excepts various speculations, and devotes his chief attention to their classification, a subject already covered sufficiently by Omeliansky, Duggeli and Bavendamm in their monographic treatment of this group of bacteria.

The author begins the monograph by stating that "the term sulphur bacteria is usually applied to the members of the group which have sulphur globules in their cells"—this is far from correct. The term "sulphur bacteria" is applied to those organisms which are able to derive their necessary energy from the oxidation of sulphur or its simple inorganic compounds. Only by overlooking this important physiological basis for defining the sulphur bacteria, can one explain how the author was led to separate the "thionic acid" bacteria and the "denitrifying bacteria" from the "sulphur bacteria," since the primary requisite for the latter is that they are able to store sulphur within their cells. The other two groups are thus considered on a par with the sulphate-reducing and saprophytic bacteria, liberating hydrogen, neglecting thereby the fact that the latter are heterotrophic bacterial forms which have nothing in common with the autotrophic bacteria.

The author states further (p. 8) that "sulphate may be reduced to elementary sulphur if the supply of oxygen be scanty," a phenomenon never demonstrated experimentally. Further "the elementary sulphur may unite with iron or other metals to form sulphides," another unproven assumption. The phenomenon most essential for the life of the sulphur bacteria, namely, the chemosynthetic utilisation of energy, is totally misunderstood. The highly hypothetical reactions and speculations suggested by Baas-Becking in explaining the mechanism of oxidation of  $H_2S$  are accepted and over-emphasised, without any critical treatment. Pleomorphism is unduly stressed; this is hardly justified for organisms that, in the great majority of cases, have not been grown in pure culture. In this connection, it may be mentioned that the author coined a new word, "pleoenergism," to designate the capacity of an organism to change its functions, as in the case of an autotrophic bacterium capable of leading both an autotrophic and heterotrophic existence; it would seem that the commonly accepted term "facultative autotrophic" would suffice for this, thus avoiding further confusion by the introduction of a new term. In order to illustrate fully the confusion introduced, in an attempt to explain the function of the  $H_2S$  in the metabolism of the sulphur bacteria, it is sufficient to cite: "It has also been shown that an undoubted gain to the bacteria follows from the assimilation of the sulphide. Hence, the advantage cannot be a direct one, and must follow because the hydrogen sulphide is a factor in a wider scale of operations than its simple decomposition." The misconceptions introduced by Molisch in the treatment of these bacteria are not only left unclarified but are even intensified. Conclusions obtained on the growth of mixed cultures of bacteria, presumably sulphur organisms, are generalised to apply to all bacteria included in this large heterogeneous group.

Among the numerous sulphur bacteria, there is one group the rôle of which in the process of sulphur oxidation has been carefully studied and is now well understood, since the organisms belonging to this group can be readily grown in pure culture, namely the *Thiobacillus* group, the first representative of which was isolated by Nathanson in 1902 and named by Beijerinck in 1904. This group is represented in nature (soil, water, dust) by a number of species, several of which have been described in detail. However, the fact that these bacteria do not contain sulphur within their cells, even if they oxidise very actively thiosulphate, hydrogen sulphide and elementary sulphur, is sufficient for the author to dispense with them as a loose group of thionic acid bacteria, one of the most important of which, namely, the *Thiobacillus thiooxidans*, is "in the strict sense not one of the sulphur bacteria." Once these bacteria are so easily disposed of, the author feels free to use their name for another quite different group, the *Bacterium bovis* Molisch. The part of the book dealing with the pigmented sulphur bacteria, which receive good treatment, has unfortunately become almost invalidated as a result of the excellent contribution of van Niel to this subject, which appeared almost simultaneously with the publication of this monograph.

In spite of the splendid illustrations, the excellent way in which the book is printed, and a fairly complete bibliography, one must conclude that an exhaustive and accurate treatment of the subject of sulphur bacteria still remains to be presented.

SELMAN A. WAKSMAN.

*The Methods of Statistics.* By L. H. C. TIPPETT, M.Sc. Pp. 222 with 10 figures. Size  $5\frac{1}{4} \times 8\frac{3}{4}$ . Williams and Norgate, Ltd., 1931. Price 15s. net.

This book is an attempt, probably destined to be one of many, to write a simple text-book on statistics, easily intelligible to the non-mathematician, but embodying the new ideas that have transformed the subject in the last quarter-century. That there is room for such a work no one will deny, but the very fact that there is a dearth of such elementary expositions, imposes a graver responsibility on the author, and should

make him all the more careful to produce a balanced and authoritative survey. Judged by this standard the present work cannot be said to be entirely satisfactory, though it may prove a useful introduction to some of the methods which have been developed by Dr R. A. Fisher, for those readers who find his own book, *Statistical Methods for Research Workers*, too difficult for first approach to the subject.

The last half of the book deals almost entirely with the analysis of variance and covariance and with the various aspects of correlation. A new line of development has been taken by introducing correlation through the medium of the analysis of variance in place of *vice versa*. This is a good feature and should do something towards destroying the excessive weight that has in the past been attached to correlation. On the other hand some of this advantage has been lost by the undue stress given to the correlation ratio, a statistic which, it might be hoped, was at last falling into disuse. Apart from this feature, however, this section of the book should form an excellent first survey of a part of statistics which is largely new. It is a pity that the chapter on the Principles of Experimental Design was not more fully developed. The concluding sentence, "thus the time to consult statistical principles is before the experiment is planned, not after the results are obtained and in confusion," is a very wise one, but it is doubtful if a single paragraph, for instance, is sufficient warning of the dangers of non-orthogonality, and the principle of making the same experiment answer several different related questions simultaneously, so successfully developed in agricultural experiments, is nowhere discussed.

The first part of the book, which deals with frequency distributions, contingency tables, and the theory of sampling, is not so satisfactory. It bears the marks of haste in its composition, and lacks consistent logical development. It is unfortunate, for instance, to find that on the first introduction of the variance no distinction is made between the true variance of the theoretical population and the estimate of variance as calculated from a sample of that population. It was just this confusion of thought which vitiated so much of the earlier work on small samples.

In general, I think, the author may claim to have succeeded inasmuch as his book is regarded as an introduction to the methods of the analysis of variance, but in attempting a comprehensive survey of the methods of statistics, such as is claimed by the title, he has attempted too much. Moreover, his omissions, from the biologist's point of view, are particularly serious. There is nowhere any mention of the method of maximum likelihood, and the discussion of discontinuous distributions is the least adequate part of the book. Nor has he realised that the more mathematics is eliminated from a work dealing with a mathematical subject the more important it is to make clear the logical basis of the subject; if this is not done the application of tests becomes a matter of mechanical routine only, and it will not be long before they are applied to data for which they are not suitable, with grievous results.

F. YATES.



## A COMPARISON BETWEEN THE EFFECTS OF AMMONIUM SULPHATE AND OTHER FORMS OF NITROGEN ON THE BOTANICAL COMPOSITION OF CLOSELY CUT TURF

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(With 7 Text-figures.)

### INTRODUCTION.

IN a previous paper<sup>(2)</sup> it was shown that the periodic application of ammonium sulphate to closely cut turf brings about a reduction in the "weeds" (*i.e.* plants other than members of the Gramineae) without producing significant changes in the soil reaction. This result was in conflict with the view held by American workers that such "weed" reduction was due to increased acidity. In the present paper experimental evidence is brought forward in support of the suggestion given in the previous paper<sup>(2)</sup>, that the weed diminution is due to a direct action of the ammonium ion. Further information has also been obtained concerning the increased reduction in the "weeds" brought about by the addition of ferrous sulphate to the ammonium sulphate.

### LAY-OUT OF EXPERIMENT.

A consideration of the possible factors that might influence the botanical composition following on the addition of ammonium sulphate to the soil, suggested that, where the changes in soil reaction were small, the herbage could only be affected differentially by the absorption of ammonium ions in the soil solution and subsequently by the absorption of the nitrate ions produced by bacterial action. It was also considered that the further botanical changes due to the addition of ferrous sulphate might be ascribed to the immediate toxicity of the ferrous ion.

With these considerations in view, the following block of plots was laid out in 1929 on closely cut turf growing in a light sandy soil. The turf was of a uniform nature and, apart from frequent cutting, had not received any fertiliser for a number of years. Eight plots in all, each  $10 \times 10$  ft., were marked out and the following treatments were applied weekly from the beginning of May until the end of September:

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Plot A. Ammonium sulphate at 3 lb. per 1000 sq. ft. (1·2 cwt. per acre).

„ B. Ferrous sulphate crystals at 4 lb. per 1000 sq. ft.

„ C. Sulphuric acid equivalent to the sulphate radicle in Plot A.

„ D. Ammonium dihydrogen phosphate equivalent to the nitrogen in Plot A.

„ E. Sodium nitrate equivalent to the nitrogen in Plot A.

„ F. Urea equivalent to the nitrogen in Plot A.

„ G. Ammonium sulphate at 3 lb. and ferrous sulphate at 4 lb. per 1000 sq. ft.

„ H. Control.

The sulphuric acid treatment was included on the ground that although in the previous experiments it had been shown that weed reduction could take place in alkaline soils, it did not necessarily follow that changes in soil reaction might not influence such botanical changes. It was not expected that the increases in hydrogen-ion concentration would be large since the soil was initially alkaline. It was thought, however, that a knowledge of the changes on the sulphuric acid plot would be of value in comparing such treatments as ammonium sulphate and sodium nitrate, since the former tends to lower the pH value and the latter to increase it. These and other factors likely to affect the growth of the plants under each treatment are shown in schematic form below.

Treatment	Possible factors
Sulphuric acid	Increase of hydrogen ions
Ammonium sulphate	„ + ammonium + nitrate ions
Ferrous sulphate	„ + toxicity due to Fe ions
Ammonium phosphate	„ + ammonium + nitrate + phosphate ions
Sodium nitrate	Increase of hydroxyl + nitrate ions
Urea	Urea + ammonium + nitrate ions.

By comparing thus the factors attributed to each treatment with the relative effects produced by these treatments, it should be possible to determine which factors are operative in producing the changes. Thus a difference between the effects of ammonium sulphate and sulphuric acid must be associated either with the ammonium ion alone or the ammonium ion in combination with the nitrate ions and not with the increase in hydrogen ions which results from both treatments.

*Mode of application.* All the materials were applied to the plots in dilute solution. It was found that the quantities required for a 100 sq. ft. plot could be conveniently dissolved in two gallons of water and the solution distributed evenly over the plot if a watering-can with a fine rose was used. In the case of sulphuric acid it was diluted to 0·1 normal by the

addition of four gallons of water, since a greater strength tended to scorch the herbage. After the applications had been made, the whole area was watered for an hour with a "sprinkler" to prevent damage due to solutions of high concentration. The first dressings were applied on May 9th and continued at weekly intervals until October 3rd.

*Technique of botanical analysis and soil sampling.* A detailed description of the method of botanical analysis has been given in the earlier paper (2). Briefly the method consists in throwing down at random a small quadrat (6 in.  $\times$  6 in.) and estimating the percentage area covered by each species. Ten or more readings are taken for an area of 100 sq. ft. and by summing these figures the mean percentage area covered by each species on the plot is obtained. The changes in botanical composition are thus expressed as changes in area covered. Where possible the data have been treated statistically.

In the previous investigation it was found that during the changes in soil reaction following manuring with ammonium sulphate, the pH varied considerably with the depth. Accordingly, plugs of soil were taken for these estimations and determinations made on each half-inch layer to a depth of three inches. The method of obtaining these plugs has been described earlier (2).

#### CHANGES IN BOTANICAL COMPOSITION.

*Changes in "total weed area."* Before detailed changes in botanical composition for each treatment are considered, a résumé of the changes in "total weed area" is, perhaps, advisable. The "total weed area" is the sum of the mean percentage areas covered by each weed species in the ten quadrats.

Table I.

Treatment	"Total weed area"		Odds that differences are not due to chance
	Initial	Final	
<i>Series I:</i>			
Control	33.1	27.3	—
Ammonium sulphate	32.9	12.2	100-1
Ammonium dihydrogen phosphate	20.2	2.8	100-1
Ammonium sulphate, ferrous sulphate	21.1	7.2	100-1
Ferrous sulphate	24.3	23.9	—
Sulphuric acid	24.5	21.0	—
Sodium nitrate	26.1	18.2	—
Urea	25.7	35.4	—
<i>Series II:</i>			
Ammonium sulphate	44.2	29.3	—
Ammonium dihydrogen phosphate	38.9	12.8	100-1
Ammonium sulphate, ferrous sulphate	44.7	14.6	100-1
			29.2



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In Table I, Series I, the initial and final weed contents between May and October are given for each treatment on this basis of "total weed area." In addition, the odds that the differences between the initial and final contents are not due to chance are shown in the last column. These odds have been calculated according to Fisher (8), using his table of "t"; where no figures are given in the table, the differences are not significant.

It is seen from Table I that while in the case of the control there is a small but not significant decrease during the season, the ammonium treatments have brought about significant and marked reductions. Sodium nitrate, sulphuric acid and ferrous sulphate also have shown some diminutions but these, as in the case of the control, are not statistically significant. Urea in contrast to all other treatments shows a stimulation of the weeds, but the increase on the plot is not in itself significant. If, however, regression equations are fitted according to Fisher's (8) methods to the curves for "total weeds" of the control, urea and sodium nitrate treatments (*vide* Figs. 1, 2, 3), it is found that there is a significant difference between the urea and sodium nitrate curves, although individually neither is significantly different from the control.

After the second botanical analysis had been made, it was realised that little information would be obtained on the comparative effect of the ammonium treatments, both on account of the very rapid weed diminution and the initial differences in the weed content of the various plots. Accordingly, in June, three new duplicate plots were incorporated in the trial. The three plots were so chosen that they contained both the same species and approximately the same contents of each species. The changes in these plots from the initial application (June 17th for the ammonium sulphate treatments and July 3rd for ammonium phosphate) until October 3rd are illustrated in Series II of Table I. The data confirm the results of the plots started in May, while in this case it would appear that under these conditions both ammonium phosphate and the mixture of ferrous and ammonium sulphates have brought about larger reductions in the area covered by weeds than sulphate of ammonia alone.

A discussion of the significance of these results is not, however, possible until the changes on each plot have been described, since it is obvious that the conception of "total weeds" as a basis for comparison is unsuitable unless the botanical composition of each plot was initially the same. On these grounds therefore the actual behaviour of the various weeds and grasses under the different treatments is first given.

*Changes in area of individual species under each treatment.* In this section the botanical changes are considered separately for each treat-

ment. The more striking fluctuations in area of the more important weed species and the "total weed" variation during the time of treatment have been illustrated graphically (Figs. 1-5). The changes in the remaining weed species and the grasses are shown in a series of tables by comparing the initial and final compositions of each plot. It is also to be noted from the tables that the initial compositions are very similar and therefore comparable.

### Series I.

*Control treatment.* In Fig. 1 the changes in percentage area during the season of the "total weeds," and for *Achillea Millefolium* and *Cerastium vulgare*, are shown as continuous lines. It is seen that the "total weed"

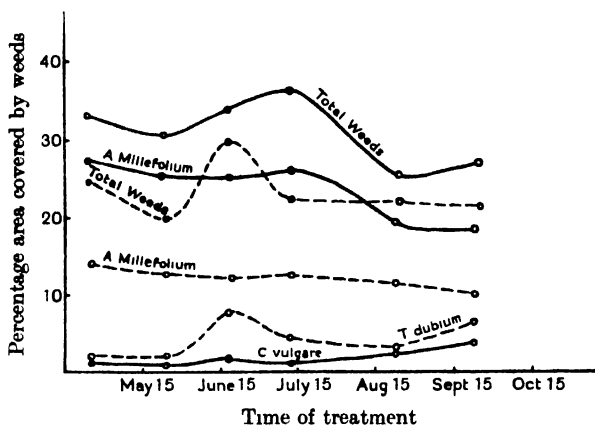


Fig. 1. Curves showing the changes in the percentage area covered with the time of treatment. The continuous lines represent the weed changes on the control and the discontinuous lines those on the sulphuric acid plot.

curve follows the fluctuations in area of *A. Millefolium*, since this is the dominant weed present. In the case of the other weeds there are, as with *C. vulgare*, small seasonal variations which are not in themselves significant. The initial and final figures for these species are given in Table II. Table II also shows that the proportions of the grass species have not altered.

*Sulphuric acid treatment.* This treatment has brought about no changes in the area covered by either the "total weeds" or by *A. Millefolium* which are significantly different from those on the control. In Fig. 1 it is seen that both curves (shown as discontinuous lines) tend to fall off during the season. On the other hand *T. dubium* has shown a significant increase, the sudden development of this species in July being reflected

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in the "total weed" curve. Of the weeds not shown graphically the changes (*vide* Table II) are not significant with the exception of the decrease of *Galium saxatile*. As in the control, the grasses on the whole have not altered appreciably, *Holcus lanatus* alone showing a significant change.

Table II.

Species	Percentage area covered					
	Control		Sulphuric acid		Ferrous sulphate	
	9. v. 29	10. x. 29	9. v. 29	10. x. 29	9. v. 29	10. x. 29
<i>Festuca ovina</i>	27.1	29.1	42.1	33.6	43.2	49.4
<i>Poa</i> spp.	29.0	30.4	28.7	34.0	25.2	21.2
<i>Agrostis tenuis</i>	2.8	4.5	1.2	6.8	0.6	1.0
<i>Holcus lanatus</i>	2.7	5.3	0.0*	3.1*	3.5	2.7
<i>Lolium perenne</i>	2.9	3.4	2.0	1.6	1.1	2.8
<i>Achillea Millefolium</i>	27.2	18.4	13.9	10.0	15.2	16.3
<i>Cerastium vulgare</i>	0.9	3.7	1.4	0.4	1.9*	0.0*
<i>Galium saxatile</i>	0.5	2.4	1.4*	0.2*	2.5*	0.3*
<i>Sagina procumbens</i>	0.3	1.3	0.6	0.5	0.7*	0.0*
<i>Lotus corniculatus</i>	0.8	0.0	1.7	2.3	—	0.6
<i>Rumex acetosa</i>	1.5	0.0	1.2	0.3	0.3	0.2
<i>Taraxacum officinale</i>	0.1	0.3	—	—	0.6	0.0
<i>Veronica agrestis</i>	—	—	0.7	—	0.3	0.0
<i>Trifolium repens</i>	0.8	0.9	0.6	0.4	1.5	0.5
<i>Trifolium dubium</i>	0.3	0.3	1.6*	6.4*	1.2*	6.0*
Bare ground	2.5	—	1.5	—	—	—
"Total weeds"	33.1	27.3	24.5	21.0	24.3	23.9

\* Statistically significant changes are marked with an asterisk.

*Ferrous sulphate treatment.* The fluctuations in area during the season for "total weeds" and *A. Millefolium* are shown in Fig. 2 as discontinuous lines. As with the two previous treatments, there have been no significant changes, but *A. Millefolium* shows no tendency to decline during the season. Significant changes have, however, taken place in some species, as shown in Table II. *C. vulgare*, *S. procumbens*, *V. agrestis* and *G. saxatile* have all decreased as against the increase of *T. dubium*. The grasses are unaffected by the treatment.

It is to be noted that ferrous sulphate has had no general depressant action on the weeds. The resistance of *T. dubium* to the ferrous ion is somewhat surprising, and is not in agreement with Ruprecht's (35) results on the susceptibility of legumes to ferrous salts.

*Urea treatment.* The changes brought about by the dressings of urea differed from those of all the other treatments in that there was a tendency for the weed population to increase. The curves for "total weeds" and for *A. Millefolium*, *G. saxatile* and *C. vulgare* are shown as continuous lines in Fig. 2. During the first three months the increase in the "total weeds" was largely made up by *A. Millefolium*, but during

August the latter started to decline and its place was taken by *G. saxatile*, which suddenly developed during this month and September. The *C. vulgare* curve has risen at a slow even rate throughout the season. None of these changes is, however, significant when the initial and final contents are compared. The general increase of the weed species is seen in Table III, although in the case of *S. procumbens* alone is this increase significant. The behaviour of the grasses differs from that of the previous treatments; the *Poa* spp. have increased and *F. ovina* decreased.

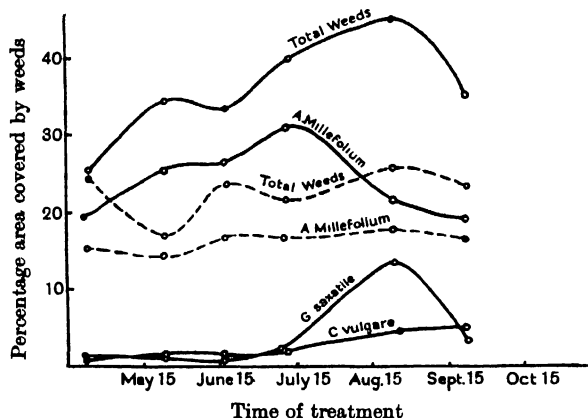


Fig. 2. Curves showing the changes in the percentage area covered with time of treatment. The continuous lines represent the weed changes brought about by urea and the discontinuous lines those produced by ferrous sulphate.

Table III.

Species	Percentage area covered					
	Urea		Sodium nitrate		Ammonium sulphate	
	9. v. 29	10. x. 29	9. v. 29	10. x. 29	9. v. 29	10. x. 29
<i>Festuca ovina</i>	40.0	20.9	43.8	19.9*	44.8*	17.0*
<i>Poa</i> spp.	28.4	42.0	15.3*	48.9*	18.7*	70.1*
<i>Agrostis tenuis</i>	1.1	0.0	0.5	0.8	0.5	0.5
<i>Holcus lanatus</i>	2.1	1.3	0.2	2.0	0.3	0.1
<i>Lolium perenne</i>	2.4	0.4	5.8	3.3	1.4*	0.2*
<i>Achillea Millefolium</i>	19.1	19.6	19.0	9.3	25.6*	1.2*
<i>Cerastium vulgare</i>	0.7	4.8	1.8	1.0	1.1	0.6
<i>Galium saxatile</i>	1.2	3.4	0.9	1.2	0.4	3.0
<i>Sagina procumbens</i>	0.6*	4.5*	1.6	5.1	0.9	6.3
<i>Lotus corniculatus</i>	0.2	0.1	0.9	0.0	1.4	0.1
<i>Rumex acetosa</i>	1.8	0.2	1.3	0.1	1.6	0.1
<i>Trifolium repens</i>	0.9	1.3	0.5	0.1	1.0	0.2
<i>Trifolium dubium</i>	0.1	1.5	0.1	0.7	0.3	0.7
<i>Taraxacum officinale</i>	1.1	0.2	—	0.7	—	—
<i>Veronica agrestis</i>	—	—	—	—	0.7*	0.0*
Bare ground	0.3	—	8.3	—	1.4	—
"Total weeds"	25.7	35.6	26.1	18.2	32.9*	12.2*

\* Statistically significant changes are marked with an asterisk.

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**Sodium nitrate treatment.** The area changes in the weed species on this plot are not significantly different from those on the control. The reduction in area of the "total weeds" and *A. Millefolium* are, however, more marked and are shown as discontinuous lines in Fig. 3. The changes in the remaining species are small; the initial and final contents are given in Table III. The grasses show the only significant changes and have reacted in a similar manner to those on the urea plot; the *Poa* spp. have increased at the expense of *F. ovina*.

**Ammonium sulphate treatment.** All the previous treatments with the exception of urea have tended to reduce the "total weed" area, but in none of these cases has the reduction been significant. Ammonium sulphate has brought about a very much larger decrease both in "total weeds"

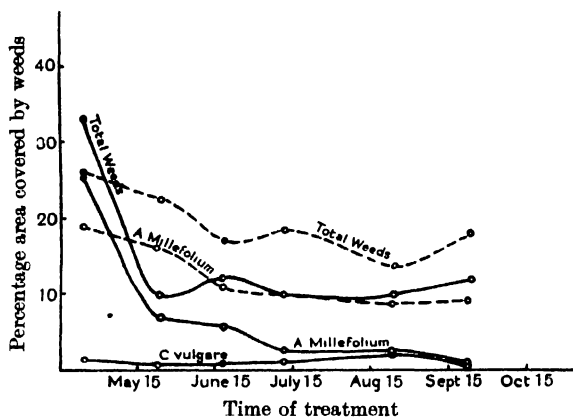


Fig. 3. Curves showing the changes in the percentage area covered with time of treatment. The continuous lines represent the weed changes brought about by ammonium sulphate and the discontinuous lines those produced by sodium nitrate.

and in *A. Millefolium*. It is seen from Fig. 3 (continuous lines) that the rates of diminution in these two cases are at first high and then gradually fall off with time. *C. vulgare* is unaffected by the treatment, while this is also the case with *S. procumbens* and *G. saxatile*, as the figures in Table III demonstrate. The other weed species, although only present in very small quantities, would appear to be susceptible to the treatment. Again, as with the other nitrogenous fertilisers, the *Poa* spp. have increased at the expense of *F. ovina*, but to an even more marked degree than in the previous treatments.

**Ammonium and ferrous sulphates.** The addition of the ferrous sulphate to the ammonium sulphate has produced little difference in the general changes. In Fig. 4 it is demonstrated by the continuous lines that there is a progressive decrease of both "total weeds" and of *A. Millefolium* during

the season. Table IV shows that the treatment tends to depress a greater proportion of the weed species than ammonium sulphate alone, only *T. dubium* out of the eleven species being resistant. It is seen that hitherto resistant species such as *G. saxatile* and *C. vulgare* have both decreased. The behaviour of the grasses is similar to that resulting from the other nitrogenous treatments; the *Poa* spp. tend to increase, *F. ovina* to diminish.

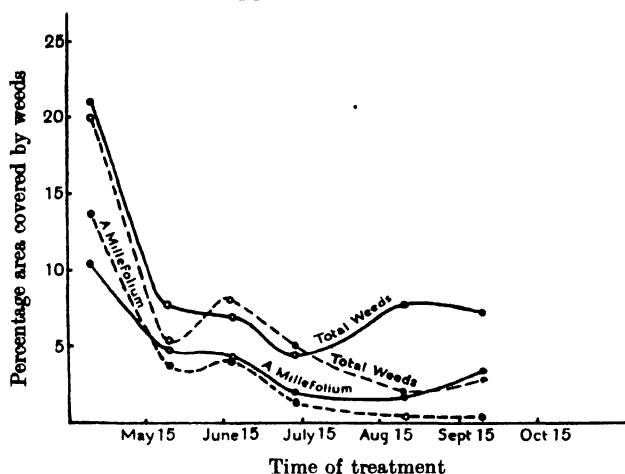


Fig. 4. Curves showing the changes in the percentage area covered with time of treatment. The continuous lines represent the weed changes brought about by a combination of ammonium and ferrous sulphates and the discontinuous lines those produced by ammonium dihydrogen phosphate.

Table IV.

Species	Percentage area covered			
	Ammonium and ferrous sulphates		Ammonium phosphate	
	9. v. 29	10. x. 29	9. v. 29	10. x. 29
<i>Festuca ovina</i>	34.0	30.2	40.8	37.4
<i>Poa</i> spp.	39.8	61.7	22.3*	49.4*
<i>Agrostis tenuis</i>	0.5	0.8	4.0	0.0
<i>Lolium perenne</i>	0.7	0.0	6.5	0.9
<i>Holcus lanatus</i>	1.6	0.0	6.2	9.5
<i>Achillea Millefolium</i>	10.5*	3.2*	13.7*	0.1*
<i>Cerastium vulgare</i>	0.2	0.0	1.0	0.2
<i>Galium saxatile</i>	2.1*	0.4*	1.0	0.0
<i>Sagina procumbens</i>	0.4	0.0	0.1	1.0
<i>Lotus corniculatus</i>	2.7	1.1	—	—
<i>Rumex acetosa</i>	1.8	0.0	2.4*	0.0*
<i>Taraxacum officinale</i>	0.7	0.0	0.1	0.2
<i>Veronica agrestis</i>	0.5*	0.0*	0.6	0.0
<i>Trifolium repens</i>	1.3	0.1	0.8	0.5
<i>Trifolium dubium</i>	0.4	2.3	0.5	0.8
<i>Ranunculus repens</i>	0.5	0.0	—	—
"Total weeds"	21.1*	7.2*	20.2*	2.8*

\* Statistically significant changes are marked with an asterisk.

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*Ammonium dihydrogen phosphate treatment.* Ammonium phosphate has brought about a control of the weeds very similar to that of ammonium sulphate. As can be seen in Fig. 4, there is a significant decrease in the discontinuous curves during the whole period both in "total weeds" and *A. Millefolium*. Of the remaining weeds the initial percentages are so small that the changes cannot be accurately determined, with the exception of *Rumex acetosa* (*vide* Table IV). The treatment has not depressed *F. ovina* but the development of the *Poa* spp. is significant.

### *Series II.*

As has already been mentioned, three additional plots were added to the trial in June, and treated respectively with ammonium phosphate,

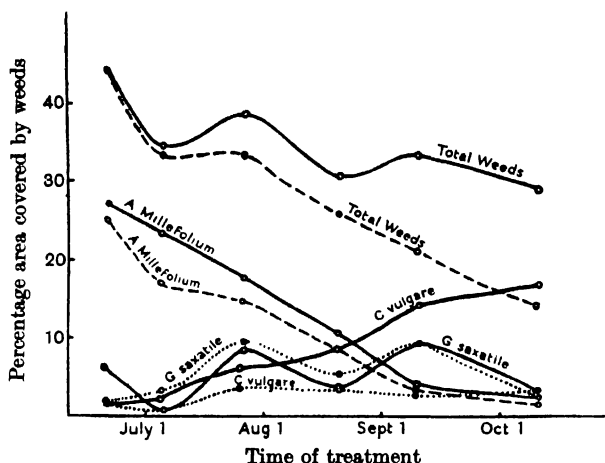


Fig. 5. Curves showing the changes in the percentage area covered with time of treatment. The continuous lines represent the weed changes produced by ammonium sulphate alone and the discontinuous lines those produced on the addition of ferrous sulphate.

and ammonium sulphate alone and in combination with ferrous sulphate. For comparative purposes these plots were so chosen that they contained initially very similar proportions of the various species; their uniformity is demonstrated in Table V.

The addition of ferrous sulphate to the ammonium sulphate under these conditions has brought differences in the behaviour of some of the weeds. With the dressings of ammonium sulphate alone it is seen from Fig. 5 that *C. vulgare* (continuous line) has increased steadily throughout the season, but that with the combination of both salts (discontinuous line) this increase has been largely reduced. On the other hand, the rate

of reduction of *A. Millefolium* has not been influenced by the presence of ferrous sulphate, while *G. saxatile* is equally resistant to both treatments. The behaviour of other species is also very similar under both treatments, the majority being depressed, although *S. procumbens* is resistant. The failure of ammonium sulphate to produce a significant decrease in the "total weeds" is thus attributable to the increase of *C. vulgare*.

Table V.

Species	Percentage area covered					
	Ammonium sulphate		Ammonium and ferrous sulphates		Ammonium phosphate	
	17. vi. 29	10. x. 29	17. vi. 29	10. x. 29	3. vii. 29	10. x. 29
<i>Festuca ovina</i>	19.5	15.3	19.7	16.3	19.6	11.1
<i>Poa</i> spp.	14.6*	49.7*	28.8*	65.6*	27.1*	61.4*
<i>Agrostis tenuis</i>	1.0	—	1.2	1.0	3.2	0.0
<i>Holcus lanatus</i>	6.2	3.6	1.0	1.4	0.9*	5.6*
<i>Lolium perenne</i>	3.8	2.1	4.5	1.1	9.9	9.0
<i>Achillea Millefolium</i>	27.1*	2.5*	25.2*	1.6*	22.3*	0.5*
<i>Cerastium vulgare</i>	1.3*	16.8*	1.5	5.9	0.2	0.6
<i>Galium saxatile</i>	5.4	4.1	2.0	3.7	4.7	2.4
<i>Sagina procumbens</i>	2.9	4.2	1.6	1.7	1.0	5.0
<i>Lotus corniculatus</i>	—	—	8.5	0.9	3.0	0.0
<i>Rumex acetosa</i>	0.4	0.1	0.2	0.1	0.4	0.1
<i>Taraxacum officinale</i>	—	—	0.6	—	0.6	0.5
<i>Veronica agrestis</i>	—	—	0.3	0.0	—	—
<i>Trifolium repens</i>	5.3	1.3	3.3	0.3	4.8	3.1
<i>Trifolium dubium</i>	1.2*	0.2*	1.5	0.3	1.7	0.6
<i>Luzula</i> sp.	0.5	—	—	—	—	—
<i>Potentilla reptans</i>	—	—	—	—	0.2	0.0
"Total weeds"	44.2	29.3	44.7*	14.6*	38.9*	12.8*

\* Statistically significant changes are marked with an asterisk.

The ammonium phosphate dressings (*vide* Table V) have again affected the different species similarly to ammonium sulphate, with the difference, however, that there has been no marked increase of *C. vulgare*.

A comparison of the botanical changes on the plots receiving their initial dressings in early May and the end of June reveals that with ammonium sulphate *C. vulgare* did not increase when the treatment was started earlier in the year. Additional information was obtained on this point, since the treatments were continued on this block of plots in the year 1930 from May till early October. In Table VI the contents of *C. vulgare* and of *S. procumbens*, after two years, are given for some of the treatments. It is seen that there has been a significant increase of *C. vulgare* with ammonium sulphate but to a less marked extent than in the "June" plot of 1929, especially when the shorter period of treatment is taken into consideration. The addition of ferrous sulphate has again prevented this increase. With *S. procumbens*, on the other hand, am-



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monium sulphate alone or in combination with ferrous sulphate would appear to have a depressant action when compared with the control changes, while ammonium phosphate as in the "June" series (Table V) has encouraged the development of this species.

Table VI.

Treatment	Percentage area covered			
	<i>Cerastium vulgare</i>		<i>Sagina procumbens</i>	
	9. v. 29	14. v. 31	9. v. 29	14. v. 31
Ammonium sulphate	0.2*	8.6*	0.9	1.6
Ammonium and ferrous sulphates	1.1	0.1	0.9	1.1
Ammonium phosphate	1.0	0.9	0.1*	9.1*
Control	0.9	3.2	0.3*	5.4*

\* Statistically significant changes are marked with an asterisk.

The plots dressed initially in June exhibit changes in the grasses similar to those on the plots started in May. The *Poa* spp. have developed, while *F. ovina* tends to decline. The significant increase in *H. lanatus* is in agreement with the May plot data.

### CHANGES IN SOIL REACTION.

For a study of the changes in soil reaction brought about by the treatments, plugs to a depth of three inches were taken from the plots before the initial dressings were applied. These plugs were divided into six transverse sections and pH determinations made by the hydroquinone method on each half-inch. In this way the relationship between soil depth and pH was obtained, as shown in Table VII. The pH figures in the table are the means of the determinations from twenty-four separate plugs. The data show that there is an increasing alkalinity with depth until the 1.5-2.0 in. layer is reached.

Table VII.

Soil depth from surface (inches)	pH	Standard error	Percentage content of calcium carbonate
0.0-0.5	7.22	±0.085	24.27
0.5-1.0	7.38	±0.05	3.53
1.0-1.5	7.55	±0.06	1.63
1.5-2.0	7.64	±0.14	1.69
2.0-2.5	7.62	±0.18	0.81
2.5-3.0	7.63	±0.08	0.81

After the final dressings had been applied, further plugs were taken from each plot and the pH determinations carried out in the same way. The changes as a result of the treatments could not, however, be ascertained by comparing directly the initial and final pH's for each plot,

since there was a seasonal decrease in alkalinity on the control plot during the experiment. On this account the effect of each treatment can only be determined by comparing the changes relative to those on the control.

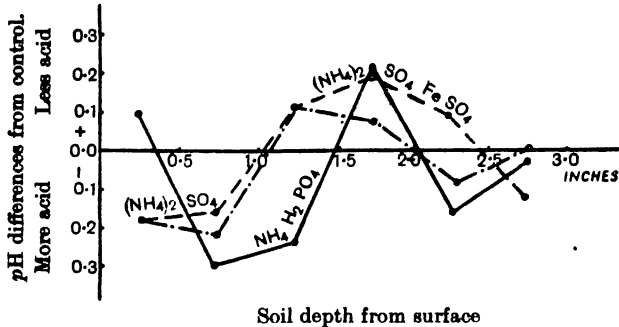


Fig. 6. Curves showing the changes in soil reaction at different depths produced by the treatments relative to the seasonal decrease in pH on the control.

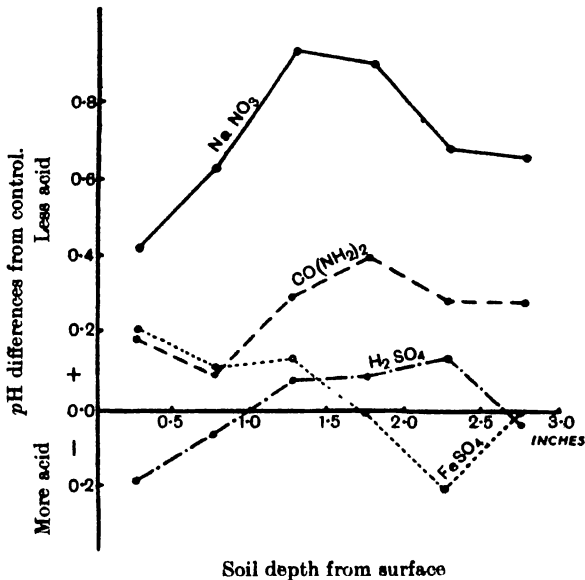


Fig. 7. Curves showing the changes in soil reaction at different depths produced by the treatments relative to the seasonal decrease in pH on the control.

In Figs. 6, 7 the differences in the changes of pH values from the control fluctuations have been plotted against soil depth. The data show that taking into consideration gradients to a depth of three inches, the changes produced by the ammonium salts, sulphuric acid and ferrous sulphate

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cannot be considered significant. On the other hand, urea and especially sodium nitrate have raised the soil pH value in the first three inches by approximately 0.25 and 0.7.

The smallness in the changes with the "acid-producing" treatments suggested that the soil contained considerable proportions of calcium carbonate still uncombined at the end of the experiment. An analysis was therefore made of plugs taken in the usual way from the control plot and the chalk content of each layer is given in Table VII. These figures show that the soil, especially in the first half-inch, is highly calcareous, while the distribution of the chalk suggests that it is due to the dressings of sea sand in previous years.

The increase in the alkalinity of the sodium nitrate plot is in agreement with the principles of base exchange, since the potentially highly alkaline sodium ions would to some extent replace the calcium ions in the soil complex. Although the data from the urea plot suggest that the soil has been rendered more alkaline, the differences are probably too small for significance.

### DISCUSSION.

The results of this experiment are in close agreement with the findings of the previous investigations that ammonium sulphate can reduce the weed population without producing any marked change in soil reaction. Furthermore, the soil reaction changes are so small that no alterations in the botanical composition would be expected from this cause, since Olsen (19) has shown for pastures that the composition remained constant unless the differences in acidity in terms of pH value were more than 0.5 to 1.0. Since this excludes the pH changes, the differences found between the effects of the various substances must be correlated either with their direct action on the plant or their indirect action through chemical changes in the soil or with both. It is suggested that the influence of the sulphate and sodium ions can be safely neglected and that the various nitrogen, ferrous and phosphate ions are alone important. The differences between ammonium sulphate and sodium nitrate treatments must therefore be attributed to the nitrogen in the former being present firstly as ammonium ions and only subsequently as nitrate ions due to bacterial action in the soil, and in the latter as nitrate ions alone.

The weed reductions brought about by the nitrogenous fertilisers cannot be correlated with the increased competitive power of the grasses since as the plots received equivalent quantities of nitrogen, similar changes should have taken place with all treatments. Competition alone cannot

explain the varying reaction of different species such as *Achillea Millefolium* and *Cerastium vulgare*. By fitting regression equations to the data in these two cases the statistical significance of the differences of behaviour with the treatments can be obtained. In the case of *A. Millefolium*, with the exception of the ammonium treatments none of the others have produced changes significantly different from the control. With *C. vulgare*, on the other hand, no treatment has brought about a significant decrease, but in contrast ammonium sulphate has significantly increased its growth; the addition, however, of ferrous sulphate has depressed the rate of increase due to ammonium sulphate so that the changes do not differ significantly from the control.

It would appear therefore that nitrogen *per se* cannot account for the various changes in botanical composition. On weeds such as *A. Millefolium* ammonium ions cause a reduction, while under the same treatment weeds like *C. vulgare* tend to increase. Urea and sodium nitrate, on the other hand, have not significantly affected the growth of either type.

Since the competition factor is ruled out and the effects on the weeds produced by sodium nitrate are not significant, the decreases brought about by ammonium compounds cannot be attributed to nitrate ions, but apparently must be correlated with some direct and differential growth depressant action of the ammonium compounds, the grasses and such weeds as *C. vulgare* being unaffected, while the majority of the weeds are susceptible and suffer poisoning as a result of the absorption of ammonium ions.

Many investigators have studied in recent years the absorption of ammonium ions and their subsequent rôle in the plant; a review of these researches is given by Onslow (20) in her book. Briefly the investigations of Beaumont (1), Loo (12), Mevius (13, 14), Naftel (17), Olsen (19), Pirschle (21), Prianishnikow (23, 26, 27, 30, 31) and Smirnow (36) have established that ammonium ions are readily absorbed by a large number of plants. Mevius (13), Engel (6, 7), Pirschle (21), Prianishnikow (31) and Hager and Stollenwerk (10) have shown in addition that the relative uptake of nitrate or ammonium ions is dependent to some extent on the hydrogen-ion concentration of the external medium, the maximum absorption of ammonium ions occurring in slightly acid solutions.

It has been demonstrated by Prianishnikow (23-31), Smirnow (36) and Mothes (15, 16) that the ammonium ions on absorption are combined with the reserve sugars to form asparagin, and that if the supply of available carbohydrates is insufficient, then the ammonium ions accumulate to a toxic extent. Plants in which this occurs are classed as "amide plants"

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by Ruhland and Wetzel(32-34), who showed in addition that there was another group, the "acid plants," in which the ammonium ions on absorption were combined with organic acids.

On this evidence the behaviour of a plant to absorbed ammonium ions would depend on two factors: carbohydrate content, organic acid content. If these criteria of ammonium ion injury are applied to the changes in botanical composition on plots treated with ammonium sulphate, then the difference in behaviour of the species must be related to their carbohydrate or organic acid content or to both. It is suggested that susceptible weeds, such as *A. Millefolium*, have a low content of both or of one of these, in contrast to the Gramineae, and resistant weeds such as *C. vulgare*, which have a high carbohydrate or acid content or both. In this connection it may be recalled that the Gramineae, in common with many other monocotyledons, store sugar in their leaves rather than starch. The weeds, on the other hand, are dicotyledons and in all probability "starch plants."

It is interesting to note that Bréal(5) and Priianishnikow(23) have shown that the Gramineae are able to withstand high concentrations of ammonium ions. Furthermore, Jones and Shive(11) found that the addition of small quantities of ferrous sulphate to two series of water cultures, one with nitrate and the other with ammonium as the source of nitrogen, produced no effect in the nitrate series but caused injurious effects in the ammonium series. This they suggested was due in some way to the iron rendering the ammonium ions toxic. This effect seems similar to the effect on the plots of ferrous sulphate in combination with ammonium sulphate. The ferrous salt applied alone had little effect on the botanical composition; mixed with ammonium sulphate the changes are more marked than with the latter alone.

While the differences between the ammonium sulphate and sodium nitrate treatments can be ascribed to the toxicity of the ammonium ion, the position is more complicated with urea, since besides the presence of ammonium and nitrate ions in the soil due to the breakdown of urea, there is the probability of direct absorption. That urea is absorbed direct is supported by the evidence of Bokorny(3), E. and G. Nicholas(18), Pirschle(22), Taubock and Klein(37) and Yamaguchi(38), but Bornemann(4), on the other hand, obtained negative results. Pirschle(22) found that with some species urea was a better source of nitrogen than ammonium nitrate but in others was inferior, while Beaumont(1) claimed that nitrates and ammonium salts gave better growth. The changes on the urea plot cannot, however, be attributed solely to direct absorption of urea, as practio-

ally all soils contain urea-splitting bacteria. Gibson(9) found that the decomposition of urea in pasture soils took place very rapidly, but in his studies the concentrations of urea used were abnormally high. Investigations in progress at this Station suggest that the decomposition of urea to ammonia and subsequently to nitrate ions by bacterial action takes place at a slow rate. The difference therefore between urea and the other nitrogenous treatments would appear to lie in the slower rate at which the ammonium and nitrate ions become available to the plant, together with the direct absorption of urea.

Apart, however, from the investigation of the mechanism by which ammonium sulphate brings about a reduction in the weeds, the findings of this experiment may have important applications in agricultural practice. It has been shown that with closely cut turf the botanical changes are governed by the chemical form of the nitrogenous fertilisers; there is the possibility that somewhat similar changes may occur in other grassland associations. It is possible that in poor hilly pastures ammonium sulphate may bring about a more rapid reduction in the weeds than other nitrogenous fertilisers, and that in a wild white clover pasture urea would be the best form of nitrogen.

#### SUMMARY.

In a previous investigation it was shown that the periodic application of ammonium sulphate to closely cut turf could bring about a reduction in the weeds (plants other than members of the Gramineae) *without producing significant changes in the soil reaction*. Evidence is produced in this investigation that such reduction is due to the direct action of the ammonium ion.

A number of small plots were laid out on a uniform piece of turf and treated, respectively, with ammonium dihydrogen phosphate, sodium nitrate, urea, dilute sulphuric acid, and ferrous sulphate alone and in combination with ammonium sulphate. The rate of application of the nitrogenous fertilisers was the equivalent of the nitrogen in 3 lb. ammonium sulphate per 1000 sq. ft. (1.2 cwt. per acre). Ferrous sulphate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) was applied at 4 lb. per 1000 sq. ft. and sulphuric acid at the equivalent of the sulphate radicle in the ammonium sulphate. The dressings were made weekly from May to September and the applications were "watered in," in order to prevent damage by solutions of high osmotic concentration.

By botanical analyses made initially and afterwards at monthly intervals the changes in the percentage area covered by each species were

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followed. It was found that a statistically significant reduction in the "total weeds," i.e. the sum of the areas covered by all the different weeds, was brought about only by the ammonium compounds. Although urea and sodium nitrate did not produce changes significantly different from the control, the slight increase brought about by urea was significantly different from the decrease with sodium nitrate.

The weeds in the sward varied in their behaviour to ammonium compounds. The majority of species, such as *Achillea Millefolium*, were reduced. On the other hand, *Cerastium vulgare* increased significantly, but the addition of ferrous sulphate inhibited this increase.

All the nitrogenous treatments produced the same changes in the grasses, the *Poa* spp. increasing at the partial expense of *Festuca ovina*. With the remaining treatments the grasses did not change.

Determinations of the pH of the soil were made at the beginning and end of the experiment. The figures showed that owing to the high chalk content there were no significant changes apart from a small increase of alkalinity caused by the sodium nitrate.

Since the ammonium compounds have alone produced significant weed diminutions, it seems evident that such reductions must be ascribed to a toxic action of the ammonium ion. There is evidence from the results of earlier workers that the absorbed ammonium ions combine with carbohydrates or with organic acids. The suggestion is therefore put forward that the differences in the response to ammonium salts of the plants of the turf may be related to differences in the content of such substances. In this view the plants resistant to the injurious action of the ammonium ions, i.e. the grasses and a few of the weeds, should show a high content of available carbohydrate or of organic acid or both. The susceptible plants—the majority of the weeds—on the other hand should show a low content.

The author wishes to thank both the Secretary of the Stoke Poges Golf Club for permission to carry out the experiment on the course, and Mr Kinnear for his help. Finally, he is indebted to Sir Frederick Keeble and Mr Page for their suggestions and to several members of the Staff at Jealott's Hill, who carried out the majority of the manurial applications.

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(Received January 28th, 1932.)



## THE EFFECT OF SEED DISINFECTION UPON THE OAT CROP IN NORTHERN IRELAND

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### INTRODUCTION.

DURING the past nine years complaints regarding the depreciation in the yield of the oat crop in Northern Ireland have been frequently encountered. In many cases an attempt has been made to raise the yield by increasing the rate of sowing and in some districts it has become usual to sow as much as 20 stones of seed per statute acre<sup>1</sup>. Every possible cause has been blamed at one time or another, but careful observation indicates that no one cause or set of conditions can be held generally responsible. When the crop follows lea, leather jackets are a common cause of serious injury, but in a large number of cases investigated it has not been possible to trace the damage to the leather jacket grub. Such diseases as smuts, leaf spot, foot-rot, sterility, seedling diseases as a whole and other more obscure physiological troubles may all on occasion play their part, but a clear conception of the rôle played by each of these factors under conditions obtaining in Northern Ireland has yet to be formed.

In 1928 a more serious outbreak of smuts occurred in varietal plots grown at the North West Agricultural School, Strabane, County Tyrone. As a result of this outbreak it was decided to conduct a series of field experiments in order to determine the relative values of seed disinfection methods from two standpoints: (1) their general effect upon the resultant crop and (2) their efficiency in securing smut control. These experiments have been in progress for the three years 1929-31 and an account of the work which has been done is given in this paper. Field facilities for carrying out this work have been freely afforded by the Agricultural Research Institute, Hillsborough, County Down, and the writers' best thanks are due to the Directors and Staff of the Institute.

### THE EXPERIMENTS.

#### *Choice of seed.*

For each year's work seed harvested from badly smutted crops was used. For 1929, samples were obtained from the Strabane plots, the varieties selected being Tyrone Tawny and Sandy, as it was in plots of

<sup>1</sup> 20 stones = 280 lb. = 126 kilogrammes. 1 acre = 0.405 hectare.

Tyrone Tawny (large grain), Sandy (large grain),  
 „ (small grain), „ (small grain).

Hamilton (B). Garron Point, County Antrim.

**Sulphur.** The seed was dusted with a reliable brand of ground sulphur such as is used for the control of powdery mildews. The sulphur was used at the rate of 3 ounces per bushel (42 lb.).

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*Copper carbonate.* The grain was dusted with a reliable brand of copper carbonate such as is used for the control of "bunt" in wheat. The material was used at the rate of 4 ounces per bushel.

*Organic mercurials.* In 1929 two dusting compounds of this type and designated as U.T. 687 and U.T. 871 were used, the grain being dusted at the rate of 4 ounces per bushel in each case. In 1930 it was learned that U.T. 687 had been marketed under the name of "Ceresan" and it was this product that was used in 1930 and 1931. U.T. 871 was abandoned after 1929. In 1931 another compound of this type known as "Abavit B" was used in addition to "Ceresan" and in this year the rate of application was reduced, each of these compounds being used at 3 ounces per bushel (= 1 ounce per stone).

*Gypsum.* Bressman<sup>(1)</sup> has recorded highly increased yields in the case of maize in West Oregon, obtained by mixing the seed with land plaster or gypsum. In order to test this method a sample of Tyrone Tawny was treated with gypsum at the rate of 1.5 lb. per bushel. To aid the adherence of the gypsum the seed was very lightly damped before treatment. This treatment was used in 1930 only.

### *Dates of treatment and sowing.*

Owing to germination injury liable to result from formaldehyde treatment, all wet treatments used in these experiments were applied the day before sowing. The dates of sowing were as follows: 4. iv. 1929, 11. iv. 1930 and 30. iv. 1931.

### *The plots.*

The field selected for the experimental plots in 1929 had been in pasture for a long period previous to 1928. Crops of rye-grass and mustard were taken in 1928, while oats and vetches formed the crop in the remainder of the field for 1929. In 1930 a site was selected in another field which had been in pasture for a long period and was to carry lea oats in that year. In 1931 the same land was used as in 1930. The type of land was of good medium loam in each case. Drilled plots only were used in 1929 and 1930, but in 1931 broadcast plots were included. The drilled plots were sown with a "Planet Junior" machine, the drills being 16 in. apart. A single plot consisted of three drills each 30 yd. long in 1929 and 40 yd. long in 1930 and 1931. The seed was sown at a depth of from 1.5 to 2 in. and the rate of sowing per drill approximated 2 cwt. per statute acre as sown by a normal farm drill with 6 in. spacing between the drills. In all cases a constant setting of the drill was used for all plots of the same

variety, so that for each variety yield comparisons could be made between the various plots. The hopper of the drill was thoroughly cleaned after each plot had been sown.

Broadcasting methods of sowing are commonly employed in Northern Ireland and in order to compare the results obtained by drilling and surface sowing a number of broadcast plots were included in 1931. Each of these plots measured 40 yd.  $\times$  2 ft. and was sown with 1.5 lb. of grain, which is equivalent to a sowing rate of 2.5 cwt. per acre. After the seed had been scattered it was covered by lightly raking the plots.

#### *Estimation of smutted panicles.*

During each season the plots were carefully searched for smutted panicles and the total number which occurred in each plot was noted. For the purpose of these experiments no differentiation is made between Loose Smut (*Ustilago Avenae* (Pers.) Jens.) and Covered Smut (*Ustilago Kollerii* Wille), the two forms being considered together.

#### *Determination of yield.*

In the case of all plots where yield estimations were made the crop was carefully cut with a reap hook and tied into sheaves. The sheaves were stooked and later removed and stored at the Plant Breeding Station, where they were to be threshed. A small bench tackle was used for the threshing and each sheaf was dealt with separately. By the use of this tackle it was possible to thresh each sheaf thoroughly and to clean out the machine after each plot was finished. The grain from each plot was weighed but no account was taken of the straw.

#### OBSERVATIONS MADE ON THE GROWING CROP.

1929.

Observations were made on the plots throughout the season and these are recorded under the headings of the different treatments employed. The order in which they are given is the order of merit in which they were placed when the plants were in the seedling stage, and which remained the same until harvest.

*Organic mercurials* (U.T. 687 and U.T. 871). By the middle of June the seedlings in these plots were larger and thicker than in any of the other plots. The difference could be observed from a distance of several hundred yards and it was obvious that for this treatment the sowing rate had been too heavy. These plots remained the best, although there was a general tendency towards the levelling up of all plots as the season

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advanced. The grain ripened from 7–10 days earlier than that of any of the other plots.

*Formaldehyde sprinkle.* These plots were placed next in order of merit. The plants were not so thick as to suggest overseeding.

*Formaldehyde steep and sulphur.* These plots were definitely not so good as those last considered.

*Controls.* The crops from these plots were very healthy but thin.

*Copper carbonate.* It appeared that this treatment adversely affected the germinating grain; the crops were poor and thin, although the individual plants were quite healthy.

*Copper sulphate sprinkle.* In these plots the germination of the grain was severely depressed. The crops were the poorest of any throughout the season and it was decided to abandon this method after 1929.

### 1930.

The differences noted between the plots treated with the organic mercurial “Ceresan” (U.T. 687 of 1929) and the remainder were not so marked as in 1929, but on the whole these plots were distinctly better than the controls. The copper carbonate and sulphur plots were distinctly poor and remained so during the whole season. The gypsum plot in the Tyrone Tawny group compared favourably with, but showed no improvement on the control. The formaldehyde steep, copper carbonate, sulphur and gypsum treatments were abandoned after this year.

### 1931.

The treatments employed in 1931 were confined to organic mercurials and the formaldehyde sprinkle. The growth differences observed were a repetition of those which were noted in 1929. In every case the crops raised from seed treated with organic mercurials showed a distinct improvement upon the controls and the formaldehyde plots. The formaldehyde plots were noticeably better than the controls. From mid-July onwards the characteristic levelling up of the plots was noted, but those raised from seed treated with organic mercurials maintained their superiority until harvest and were ready for cutting from 7–10 days before the remainder.

### RESULTS.

#### *Germination capacity under laboratory conditions (1929).*

In order to determine the effect of treatment upon the germination capacity of the seed under laboratory conditions, in 1929 a sample of each lot was submitted to the Seed Testing Station. Germination tests were carried out in clean Petri dishes lined with filter paper moistened with

tap water. In the case of each test, four lots of 100 seeds were incubated at approximately 22° C. for 10 days, when germination counts were concluded. In the case of the samples which had been treated with copper sulphate and where the germination capacity had been adversely affected, the period was extended to 14 days, in order to give the grain every chance. The germination tests were carried out over a period of two months, four being made in all, the first as soon as possible after treatment, the second after an interval of a fortnight, and the third and fourth at approximately monthly intervals after the second. The dates of testing were: 9. iv. 1929, 23. iv. 1929, 21. v. 1929, 18. vi. 1929. As in no case did the lapse of time after treatment appear to affect the germination capacity, the results from the four tests have been averaged and the average capacities for germination are given in Table I. The procurement of each of the figures given involved the testing of 1600 seeds.

Table I.

	Tyrone lawny				Sandy			
	Large grain		Small grain		Large grain		Small grain	
	Average germination capacity %	Total no. of grains which developed mould	Average germination capacity %	Total no. of grains which developed mould	Average germination capacity %	Total no. of grains which developed mould	Average germination capacity %	Total no. of grains which developed mould
Control	98.25	2	95.25	5	97.0	7	93.25	4
U.T. 687	98.25	0	97.25	0	—	—	—	—
U.T. 871	—	—	—	—	88.25	3	83.50	5
Formaldehyde sprinkle	96.00	5	94.75	5	94.0	13	94.25	9
Formaldehyde steep	96.00	6	91.25	13	93.5	16	90.50	16
Sulphur	—	—	97.25	1	90.5	6	94.00	5
Copper carbonate	97.50	0	95.25	1	93.5	7	93.75	7
Copper sulphate sprinkle	60.00	1	60.25	2	63.75	10	76.00	12

From Table I it is seen that the only treatment which seriously depressed the germination of the seed was the copper sulphate sprinkle, where in every case the figure was reduced by approximately 30 per cent. This depression remained approximately constant for the whole period during which germination tests were in progress. The only other significant difference occurred in the case of Sandy oats dressed with U.T. 871, where a depression of germination of approximately 10 per cent. was registered. In the case of U.T. 687 and all other treatments the germination capacity remained normal. The slight depression which occurred after formaldehyde treatment is represented by the higher percentage of moulded grains consequent upon such treatments.



been harvested from a badly infected crop, yet smuts occurred to a negligible extent in a large number of the plots. Formaldehyde sprinkle gave complete control in nearly all cases, while "Ceresan" and U.T. 871 proved to be almost as satisfactory. The slightly poorer control given by "Ceresan" in 1931 may be due to the fact that the amount of the material used for this season was reduced from 4 ounces to 3 ounces per bushel of seed, while it is also to be noted that smut infections were heavier for this year. Unfortunately smuts occurred to a negligible extent in the plots sown with seed treated with "Abavit B" and it is not possible to arrive at the value of this material for smut control from the results obtained in 1931. Formaldehyde steep was quite as satisfactory but was abandoned after 1930 in favour of the sprinkle process, which is so much more easily carried out. The copper sulphate and copper carbonate treatments exerted some measure of control but proved to be inferior to formaldehyde and organic mercurials. Copper treatments were abandoned after 1929 on account of their adverse effect on the resultant crop in that year. Sulphur appeared to be useless as a fungicide for smuts and was abandoned after 1930. Gypsum did not control smuts in the Tyrone Tawny plots of 1930 and was also abandoned. Methods of drilling and broadcasting *as employed* in 1931 do not appear to have influenced the intensity of the smut attack.

#### *The yield of grain.*

In 1929 and 1931 some or all of the plots were examined for yield. The growth in each plot was very uniform and no factor was observed which would have been likely to affect the reliability of the results. In 1930 the crop, which followed lea, was not uniform and no yield determinations were made.

The results are recorded in Table III, and the outstanding feature shown is the large increase in yield consequent upon treatment with organic mercurials. In one case only was no increase obtained, viz. that of Tyrone Tawny in 1931, and the slight decrease registered in this case (2.8 per cent.) is probably not significant. It is also to be noted in the case of this particular variety that the grain ripened at least three weeks before the remainder, and as all the plots were harvested at the same time considerable "shelling out" of ripe grain had occurred. From the remaining ten experiments where yield determinations were made, the "Ceresan" plots gave an average increase in grain yield of 44.8 per cent. above that of the controls. Two yield determinations were made from "Abavit B" plots in 1931 and the average increase in grain yield



Table III.

*Giving the yield of grain from each plot when yield estimations were made.*

1929	Tyrone tawny (large grain)				Tyrone tawny (small grain)				Sandy (large grain)				Sandy (small grain)			
	Yield		Increase or decrease %		Yield		Increase or decrease %		Yield		Increase or decrease %		Yield		Increase or decrease %	
	lb.	oz.			lb.	oz.			lb.	oz.			lb.	oz.		
Control	9	13	—		10	1-25	—		12	0	—		10	3-75	—	
U.T. 687	12	15	+ 3-9		14	10	+ 45-3		Not done				Not done			
U.T. 871	Not done		—		Not done		—		16	5-5	+ 35-9		16	2-75	+ 57-9	
Formaldehyde sprinkle	10	6-5	+ 6-0		11	4-75	+ 12-1		15	4-75	+ 27-6		12	7-75	+ 22-0	
Formaldehyde steep	11	2	+ 13-4		9	15-25	— 0-6		11	10	— 3-1		9	5	— 9-1	
Sulphur (dusted)	Not done		—		8	14	— 11-8		12	9	+ 4-7		9	12-75	— 4-3	
Copper carbonate (dusted)	8	12	— 10-8		9	14-75	— 1-2		8	15	— 25-5		8	3-5	— 20-1	
Copper sulphate sprinkle	6	7-25	— 34-2		9	2-75	— 9-0		10	6-25	— 13-5		9	6-75	— 7-9	
1931	Potato A				Potato B				Tyrone tawny				Tam Finlay (McGill Smith)			
	Yield		Increase or decrease %		Yield		Increase or decrease %		Yield		Increase or decrease %		Yield		Increase or decrease %	
	lb.	oz.			lb.	oz.			lb.	oz.			lb.	oz.		
Drilled	10	8	—		12	7-5	—		15	5-5	—		8	6	—	
Control	16	12	+ 59-5		17	8	+ 40-2		10	9	+ 26-1		10	9-5	—	
Ceresan	17	3-5	+ 64-0		17	7	+ 37-7		14	14	—		14	12	+ 30-6	
Abavit B.	14	10	+ 39-3		12	9-5	+ 1-0		14	2	— 7-7		11	3-5	+ 5-9	
Formaldehyde sprinkle																
Broadcast	8	0	—		8	1	—		—		—		—		—	
Control	12	7-5	+ 55-9		10	6	+ 28-7		—		—		—		—	
Ceresan	9	12-5	+ 22-3		8	8	+ 5-4		—		—		—		—	
Formaldehyde sprinkle									—		—		—		—	

registered in this case was 50·8 per cent. Although the formaldehyde sprinkle treatment gave excellent results from the point of view of smut control, yet the average increase in grain yield obtained in the same ten experiments was only 16·0 per cent. In one case in 1929 the formaldehyde steep method gave an increase of 13·4 per cent. of grain, but in the three other experiments carried out in that year a slight decrease was registered. This difference occurring in the use of the two formaldehyde methods may be due to germination injury resulting from the steeping method. Sulphur did not appear to produce any significant effect upon the yield of grain. In the case of both copper sulphate and copper carbonate a significant decrease in yield occurred.

Treatment with organic mercurials gave similar results in both drilled and broadcast plots in these experiments.

#### LARGE SCALE EXPERIMENTS.

After such interesting results had been obtained from the small scale plots in 1929 it was decided to conduct a large scale experiment at Hillsborough in 1930. This experiment entailed two plots, each one statute acre in area and selected in a portion of a field where the soil characteristics were uniform. One plot was sown with untreated seed and the other with seed dressed with "Ceresan" at the rate of 4 ounces per bushel. The seed used was pure line Potato and had been saved from a smut free crop. Treatment was carried out on 29. iii. 1930 and the seed was sown (drilled) on 7. iv. 1930 at the rate of 18 stones per statute acre.

During the growth of the plots throughout the season, the crop from treated seed appeared to be better than that from untreated seed. Although both plots were harvested on the same day (5. ix. 1930), the grain of the treated plot was ripe from 7-10 days earlier than that of the untreated. The produce from each plot was stacked separately and the stacks were threshed on 20. x. 1930. The yields of grain were as follows:

Untreated seed	9 cwt. 1 qr. 7 lb.
Treated seed	13 cwt. 1. qr. 23 lb.

This result represents an increase of 43·1 per cent. in grain yield. A series of large scale experiments was commenced throughout Northern Ireland in 1931 and it is hoped to publish the results from these in due course.

## GENERAL DISCUSSION.

More vigorous growth in the earlier stages of the crop and substantial increases in the yields of various crops as a result of seed disinfection with organic mercurials have been recorded in many countries. Apart from their value as fungicides it has also been claimed that organic mercury compounds actually stimulate plant growth. Sampson and Davies(5), however, were unable to demonstrate any actual stimulatory effect and concluded that for the present their action can only be regarded as fungicidal. One interesting result emerging from the experiments here described is the fact that, whereas seed treatment with formaldehyde gave, on the whole, slightly better results with regard to the control of oat smuts (as estimated by the occurrence of smutted panicles), yet the average increase in grain yield was only 16 per cent. as against 44.8 per cent. resulting from the use of organic mercurials. Germination tests in the laboratory showed that the formaldehyde sprinkle treatment did not depress the germination capacity, but they did suggest that seed which has been so treated is more liable to become moulded than untreated seed. On the other hand, seed dusted with organic mercurials, and thus retaining a coating of the active fungicide, remained very free from moulds.

In the case of many crop diseases which are seed borne, portions of the parent plant such as testa, pales, etc. which go to make up the "seed" frequently provide a foothold from which the parasite may attack the seedling, while at the same time they offer a suitable feeding ground for soil organisms which, particularly under unfavourable germination conditions for the seed, may thus become established as parasites. The wet cold springs which frequently occur in Northern Ireland provide such conditions. Seed which has been treated with formaldehyde retains none of the fungicide at the time of sowing and the sterilised pales, etc. provide a suitable pabulum for the growth of any trace of mycelium, etc. which may have escaped at the time of treatment, and also for the growth of organisms present in the soil. Organic mercurials, on the other hand, remain effective after the seed is sown and would appear to provide an efficient barrier against these sources of infection. The young seedling is thereby effectively shielded during its early stages of growth and a better established plant is produced. O'Brien and Prentice(2) obtained a better establishment of oat seedlings in field plots after treatment with organic mercurials than after the use of formaldehyde, and similar evidence was obtained in the experiments under review.

## SUMMARY.

1. This paper deals with a series of experiments carried out in Northern Ireland over a period of three years in order to determine the value of various seed treatments for controlling smuts and for use as general seed disinfectants in connection with the cultivation of the oat crop.

2. In so far as the control of oat smuts is concerned and at the concentrations used in these experiments, copper sulphate and copper carbonate proved to be of some value but caused definite injury to the crop, while sulphur and gypsum proved to have no fungicidal value. Formaldehyde gave complete control in nearly every case, the sprinkle method being considered more satisfactory than steeping, as it is more easily carried out and as the results obtained indicate that in some cases steeping may cause crop injury. The organic mercurial—"Ceresan"—gave almost as good results as formaldehyde.

3. As general seed disinfectants the organic mercurials—"Ceresan" and "Abavit B"—proved to be more satisfactory than formaldehyde, the average increase in grain yield from these plots being approximately 30 per cent. above the yield from the formaldehyde plots which in turn showed a yield increase of 16 per cent above the controls.

4. It is suggested that the greater efficiency of organic mercurials is due to the fact that the fungicide is retained by the grain at the time of sowing and remains operative during the early stages of crop development without causing injury to the plants.

The fact that oat grain has been treated with "Ceresan" and "Abavit B" at varying intervals before sowing with complete success indicates that this method of treatment possesses a distinct advantage over the formaldehyde method where, in order to preclude possibly injury during germination, it is necessary to carry out the treatment immediately prior to sowing. No causes of poisoning as a result of the use of organic mercurials have occurred so far and it is possible that by taking reasonable precaution the use of these compounds for seed dressing may be safely advocated.

## ACKNOWLEDGMENTS.

The writers' thanks are due to the staff of the Seed Testing Station for carrying out the germination tests made during these experiments, to the staff of the Plant Breeding Station for the excellent facilities afforded during threshing operations, and to the firms which kindly supplied the "Ceresan" and "Abavit B" for experimental purposes.

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(Received February 25th, 1932.)

# THE *FUSARIUM* BULB ROT OF NARCISSUS

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(With Plate XXVII and 2 Text-figures.)

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## I. INTRODUCTORY.

THE Narcissus plant in cultivation shows a considerable number of diseases affecting the underground parts. There still exists considerable uncertainty with regard to the etiology of some of these diseases which is reflected in a good deal of confusion in the nomenclature. As the present paper deals with one type of bulb disease, it will aid clearness to give a list of the other diseases affecting the underground parts of Narcissus, together with a brief description of the symptoms of each, compiled from published accounts and from observations made during the present work.

(a) *Eelworm disease* due to a biologic race of *Tylenchus dipsaci* (*T. devastatrix*); easily recognisable by the presence of these nematodes, usually in large numbers, in the affected bulb scales. Characteristic swellings are visible on the leaf parts, which in advanced stages of the disease are typically severely distorted and dwarfed. The pathogenicity of *T. dipsaci* was proved by J. K. Ramsbottom<sup>(15)</sup> in England, and by van Slogteren<sup>(16)</sup> in Holland. The bulb-rotting eelworm may often be accompanied in the decayed tissues by other organisms, such as various fungi, saprophytic eelworms and mites. Thus Welsford<sup>(22)</sup> describes a case of bulb infection in which the eelworm *T. dipsaci* was associated with a fungus stated, without description, to be *Fusarium bulbigenum*. The eelworm was considered by her to be the active cause of the disease and the fungus to be merely saprophytic. The question of the discrepancy between Welsford's and the present writer's findings with regard to the *Fusarium* will be discussed later in this paper.

(b) *Diseases due to insects*. Three species of fly are found attacking bulbs in this country, *Meredon equestris*, *Eumerus strigatus* and *E. tuberculatus*. *Meredon* is undoubtedly a primary parasite, but there has been controversy as to whether the *Eumerus* spp. attack healthy bulbs, the balance of opinion at present being that they can do so. Fly attack can be distinguished by the presence of the larvae embedded in a mass of brown pulp in a cavity in the bulb.

(c) *Disease due to Botrytis narcissicola*. The black sclerotia of this fungus may be found on the papery bulb scales after lifting, but the bulb itself is not as a rule attacked during summer storage. If storage is prolonged into the autumn, as often happens with retail stocks, the fungus may be found slowly attacking the base, and finally the scales. Rotting due to *Botrytis narcissicola* may be distinguished by the following characters: the frequent presence of black sclerotia on the papery bulb scales; the yellow-brown colour of rotted base and scale tissue, as compared with the chocolate or reddish brown caused by *F. bulbigenum*; and eventually by the presence of large flat black sclerotia embedded in the rotted fleshy scales.

Inoculation experiments carried out by the writer with bulbs of the variety "Emperor" showed that *B. narcissicola* is a definite though a weak parasite. The fact that its optimum for growth lies near 20° C., as contrasted with an optimum of approximately 30° C. for *F. bulbigenum*, may possibly explain why this fungus does no appreciable damage during the summer months.

(d) *Basal rot or root-plate rot* of Beaumont<sup>(1, 2)</sup> is distinguished by

"the dry brown decay of the root-plate while the scales remain healthy." Roots are few or entirely wanting. According to Beaumont<sup>1</sup> and the writer's own observations, affected plants show a dwarfing of the leaves and flower stalk, and a diminution in flower size. Beaumont further reports the frequent association of the fungus *Ramularia macrospora* (syn. *Cylindrocarpon radiculicola* Wr.) with such diseased bulbs, but so far a causal relationship has not been proved. The disease appears to be widely spread in England, the writer having noted it in Cornwall, Isles of Scilly, Lincolnshire, and having received specimens from the London district.

Basal rot or root-plate rot, as described by Beaumont, appears to be very similar to, if not identical with, a disease described under the same name by Wooley Dod<sup>(6)</sup> in 1894, and attributed by him to excessive nitrogenous fertilisation. Whether roots are formed and subsequently rotted as claimed by Dod, or whether they are never formed at all, requires further investigation. Crawford<sup>(5)</sup> identified with Dod's "basal rot" a condition which he believed to be due to poisoning of the bulbs by ammonium compounds in the soil, and their subsequent infection with *Penicillium* from the same source.

Weiss<sup>(19)</sup>, McWhorter<sup>(13)</sup> and other American writers use the term "basal rot" for a disease of storage in which the causal organism is a *Fusarium*, and in which the whole bulb may be involved. This disease appears to be identical with the subject of the present paper. While this loose usage of the term "basal rot" is likely to lead to some confusion, it appears to be so widespread that it will probably persist.

(e) *Root rot*. Rotting of roots of Narcissus plants has been described in Holland by Gerretson, Hissinck, Volkersz and Zijlstra<sup>(10)</sup> and others, in U.S.A. by Wedgworth<sup>(18)</sup>, and briefly referred to in England by Beaumont<sup>(2)</sup>. As a rule only root tissue is involved, the base and scales remaining unaffected, though the size of the shoot may be reduced. The affected roots have elongated brown streaks on the white surface, or they may be healthy for a short distance at the base, but become hollow near the tip from the breakdown of the cortex.

Through the courtesy of Mr A. Beaumont and Mr G. W. Gibson respectively the writer has collected material of this disease in Cornwall and Scilly. Microscopic examination of affected root tissue showed the presence of pale brown bodies which in size, colour, shape and arrangement resembled the chlamydospores of *Cylindrocarpon radiculicola* Wr. (*Ramularia macrospora* Wr. non Fres.). This fungus was repeatedly

<sup>1</sup> Verbal statement.



isolated from specimens without difficulty. In a recent paper Feekes<sup>(8)</sup> describes the infection and subsequent rotting of the roots of *Narcissus* bulbs when planted in field and greenhouse soil which had been inoculated with cultures of *Cylindrocarpon radiculicola* Wr.

Whether there is any connection between Root Rot and the root-plate rot described by Beaumont, how far both or either may be due to purely physiological causes, and whether possibly some rôle is to be assigned to *F. bulbigenum* in connection with either of these diseases are points that require further work.

## II. HISTORICAL REVIEW OF THE *FUSARIUM* ROT PROBLEM.

The first record of the association of *F. bulbigenum* with a disease of *Narcissus* bulbs is believed to be that of Jacob<sup>(12)</sup> who described rotting of the base and scales of stored bulbs as a characteristic feature.

During the same hot summer (1911), Westerdijk<sup>(23)</sup> records a rot of *Narcissus* bulbs beginning in the basal cushion, and due to a *Fusarium* of the type *Elegans*.

Massee<sup>(14)</sup> describes a disease of *Narcissus* bulbs due to *F. bulbigenum* Cke. & Mass., forming on the leaves yellowish spots covered with spores, and passing down to the bulb where it spreads, assisted by such saprophytes as *Penicillium* and eelworms. Chlamydo-spores are stated to be present in the leaves and scales, and on germination these produce elliptical secondary spores in chains which infect the young leaves in spring, after which the disease is spread by spores of the *Fusarium* type. Westerdijk<sup>(24, 25)</sup>, commenting on Massee's paper, says that *Fusarium* fructifications are seldom, and chlamydo-spores never, observed on the leaves, and concludes that the bulb scales first become infected when the leaves are already dead. She attributes the symptoms described by Massee to a mixture of two diseases, viz. to eelworms producing a leaf spotting, and *Fusarium* rotting the bulbs. It will be indicated later that the condition described by Massee may be due in part to the presence of a third organism. Westerdijk obtained successful inoculations with two fungi isolated from diseased bulbs, *F. bulbigenum* and "*F. gemmiperda*," two species which are stated to differ only in that the chlamydo-spores of the latter are blue in the mass (Westerdijk<sup>(25)</sup>). The latter may have been an erroneous determination as Wollenweber<sup>(26)</sup> gives *F. gemmiperda* Aderh. as synonymous with *F. lateritium* Nees. var. *fructigenum* (Fr.) Wr., in which chlamydo-spores are absent.

More recently a bulb rot attributed to *F. orthoceras* App. and Wr. has been studied in Holland by Feekes<sup>(8)</sup>. In this paper the spread of the

fungus within the bulb tissues is described in considerable detail. Bulbs planted in unsterilised soil infected with *F. bulbigenum* remained healthy, but where the soil was infected with *F. orthoceras* symptoms regarded as an early stage of *Fusarium* attack developed in some of the bulbs. These however, did not subsequently become diseased in the typical manner. Inoculations of stored bulbs with *F. orthoceras* gave successful infections and the organism was readily reisolated. Further work will show whether bulb rot is due more commonly to *F. orthoceras* or to *F. bulbigenum* as found by the present writer.

Further references serving to indicate the wide geographical range of the disease are those of Drayton<sup>(7)</sup> from Canada, of an anonymous writer<sup>(27)</sup> (Gram and Weber?) from Denmark, and of Beaumont<sup>(1, 2)</sup> from England. Beaumont records the association of the following fungi with *Fusarium* rotted bulbs: *F. moniliforme* Sheldon, or its variety *maius* Wr. and Reink.; *F. solani* App. and Wr.; *F. bulbigenum* Cke. and Mass.; *Cylindrocarpon album* (Sacc.) Wr.; *Ramularia macrospora* Fres.; and *Fusarium* spp.

### III. EXPERIMENTAL.

This investigation began from the standpoint that, while the eelworm disease of Narcissus bulbs was definitely recognised, it was not certain whether the frequent association of *Fusarium* with decayed bulbs was of importance. The first step therefore was to make a survey of the organisms occurring in rotted bulbs, paying special attention to *Fusarium* species, and to make inoculation experiments with such *Fusaria* isolated.

#### A. OCCURRENCE AND SYMPTOMS OF THE DISEASE.

##### (i) *In the store.*

The first external sign of disease of bulbs in storage is usually a brown discoloration of the region of the basal ring, the white tissue between the lowest papery scales and the root zone. This part of the bulb becomes soft and the rot begins to spread through the base and up the scales (Plate XXVII, fig. 1). Softening is accompanied by a pronounced dark discoloration of the affected tissue, usually a chocolate-brown, but sometimes a brick-red, or even a bright violet in certain bulbs of the variety "Loveliness." Eventually the whole bulb becomes rotted. The scales, however, do not become mushy as they do when attacked by fly or eelworm, and eventually the bulb dries up to a hard shrivelled mummy. White mycelium, bearing abundant microconidia, is present between the

rotted scales, but few septate *Fusarium* spores are formed there. Chlamydospores are not always formed in the bulb as invariably they are in culture. On the basal ring is developed a dense mass of white mycelium, usually of the *Fusarium* (Plate XXVII, fig. 2), but under humid conditions of storage this may consist of *Trichoderma viride*.

(ii) *In the field.*

Although rotting caused by *F. bulbigenum* is best known among dormant stored bulbs, from the following observations it is believed to occur in the field.

A stock of the white trumpet daffodil "Peter Barr" had been hot-water treated in the summer of 1929 two or three weeks before planting. During this interval a number of the bulbs rotted and a culture of *F. bulbigenum* from one of them proved to be pathogenic to single bulb scales when tested by the method to be described below. In April 1930 when an examination was made of the growing stock, a considerable proportion of the bulbs had "missed." The missing bulbs when dug up were found as a few shreds of blackened, almost unrecognisable tissue, or as bulbs showing a brown rot of base and scales when cut open, with perhaps a small amount of normal white tissue. It is considered probable that in these bulbs infection was active at the time of planting. Only apparently healthy bulbs were believed to have been planted but, in view of the difficulty of detecting early stages of attack, it need not be concluded that all "missing" bulbs had become infected subsequently to planting.

Besides "misses" there were many plants with a poorly developed shoot, consisting of one or two narrow yellowish leaves arising from a bulb with perhaps the basal tissue and the lower portions of several inner fleshy scales rotted a dark chocolate-brown. The roots, if one or two were present, appeared normal (Plate XXVII, figs. 3, 4). *F. bulbigenum* was isolated from bulbs in this condition during April. Affected bulbs were not seen to flower.

When the stock was lifted at the beginning of July about 170 bulbs rejected by the grower were removed for examination. More than half of these contained larvae of *Eumerus* sp. which must have entered the bulbs while in the ground. Of the bulbs not attacked by fly larvae, thirty-seven which were still in an early stage of attack had produced roots and shoot during the current year, a fact which is interpreted to mean that rotting had begun during that summer, and had not been active at the time of planting—in contrast to those bulbs "missing" in

the spring. Twenty-five per cent. of the apparently sound bulbs harvested became rotted before the end of August, after which no further rotting occurred, and in 1931 no disease or missing bulbs were evident in the growing stock. During storage in the summer of 1931 disease appeared sporadically in a few bulbs. Throughout the period described *Fusarium* was observed in the affected bulbs, and isolations made at intervals yielded the typical *F. bulbigenum* usually associated with the bulb rot. Two of these strains were tested for pathogenicity and proved to be active. These facts are taken as evidence that the disease was largely due to *F. bulbigenum* during the twelve months following upon hot-water treatment.

A condition of the plant which results in much "splitting" of the bulb and which was observed in an infected stock of the variety "Victoria" may be due to a slight attack by *Fusarium* which the bulb was able to check. When planted in the autumn of 1930 many of the bulbs showed patches of decay in the neighbourhood of the basal ring. In the following spring, instead of producing the normal leaves only, the bulb sent up some ten or twenty narrow grass-like leaves, which were however green and healthy in appearance. Examination of some of these bulbs at the time showed that, on the side of the plant whence the abnormal foliage arose, part of the basal cushion was missing. When lifted in the summer these bulbs were found to be more or less surrounded by a sheathing layer of narrow secondary bulbs tightly packed round the primary. It appears possible, therefore, that incipient *Fusarium* attack may be one cause of excessive splitting of the bulbs, though definite evidence of this has still to be obtained.

#### B. ISOLATIONS FROM DISEASED BULBS.

Isolations were made not only from diseased bulbs in a fairly advanced state of rotting, and having the typical symptoms outlined above, but also from others which were in early stages of attack or which showed doubtful symptoms. The purpose of these latter isolations was to attempt to discover the earliest stages of infection. An artificial selection was thus introduced into the following list of fungi isolated, which tends to mask the very frequent association of *F. bulbigenum* with bulb rot. In making isolations the bulb was sliced longitudinally, then in order to avoid organisms introduced from the exterior of the bulb on the knife, the tops of some of the exposed scales were pulled forward and a piece of tissue removed from the edge of the discoloured area from the middle of a scale. The tissue was placed on nutrient agar. Apple, plum or acidified

potato-extract agars proved most useful in making the preliminary isolations from decayed tissue. Most of the bulbs from which isolations were made were examined for the presence of *Tylenchus dipsaci*, which was rarely observed. A considerable number of organisms appeared during the course of this work, as may be seen from Table I.

Table I.

Organism	No. of bulbs from which isolated
<i>F. bulbigenum</i> Cke. and Mass.	116
<i>F. moniliforme</i> Sheldon	20
<i>F. culmorum</i> (W.G. Sm.) Sacc.	3
<i>Fusarium</i> spp.	3
<i>Cylindrocarpon radiculicola</i> Wr. ( <i>Ramularia macrospora</i> Wr. non Fres.)	24
<i>Cylindrocarpon</i> sp.	3
<i>Phoma</i> sp.	6
<i>Botrytis narcissicola</i> Kleb.	15
<i>Botrytis</i> sp.	21
<i>Stagonospora Curtistii</i> (Berk.) Sacc.	2
<i>Pythium</i> sp.	6
<i>Sclerotium</i> spp.	18
<i>Trichoderma viride</i> ( <i>T. lignorum</i> , <i>Eidamia viridescens</i> )	72

In addition there appeared numerous organisms such as species of *Penicillium*, *Aspergillus*, *Mucor*, *Rhizopus*, *Alternaria*, etc., which were not regarded.

Table I shows the very frequent association of *F. bulbigenum* with Narcissus bulb rot. The two fungi *F. moniliforme* and *Cylindrocarpon radiculicola* also occur with considerable frequency. The remainder, though some of them are likewise of frequent occurrence (e.g. *Trichoderma viride*) may be dismissed in the present connection. In view of the statements in the literature (see above, p. 479) regarding the fungi *F. bulbigenum*, *F. moniliforme* and *Cylindrocarpon radiculicola* attention was specially directed to them. The following brief descriptions of these three fungi apply to strains isolated and studied during the course of the work.

(i) *F. bulbigenum* Cke. and Mass.

The cultures which have been isolated fall broadly into two groups which will be referred to as strains A and B. Whether a finer morphological grouping could be established was not determined. The following description refers to strain A.

(a) *General cultural features.*

*Aerial mycelium* white to salmon-pink on a variety of agar media.

*Colour production in the medium.* On oatmeal, potato and malt extract agars the medium is frequently uncoloured, but may acquire various shades between pink and bluish; a deep blue coloration common over small areas near the base of the slant, where it may or may not be associated with the presence of sclerotia; on acid media frequently pinkish; on Brown's synthetic medium as modified for colour production (3), usually blue or violet; on steamed rice grains bright salmon-pink to pale violet, but no benzolic odour so far observed.

*Sclerotia* have been noted in cultures on potato and oatmeal agars; white or buff when young, but may become bluish later; distributed over the surface of the medium, but specially numerous in somewhat dried-up cultures, where they are formed freely along the region where the culture medium was in contact with the glass tube.

*Sectoring* of pure plate cultures was observed, but the question of saltation was not pursued. In different isolations some variation was noted; e.g. in macroconidial dimensions, intensity of colour production and in abundance of macroconidia, sclerotia and chlamydospores.

*Optimum temperature* for growth appears to be just below 30° C.

(b) *Microscopic features.*

*Microconidia*: 0-1 septate, oval or slightly curved, apedicellate, more abundant than the macroconidia both in culture and in the bulb; formed abundantly in the aerial mycelium and in the medium in false heads (*Cephalosporium*), and have not so far been observed in chains.

	Average
Dimensions: 0-septate, 3-20 × 2-3.5 μ	7 × 3 μ
1-septate, 10-30 × 2-3.5 μ	14 × 3 μ

*Macroconidia*: found sparingly in the aerial mycelium where they tend to be degenerate in form; occasionally malt or potato extract agar tube cultures have been found producing well-formed, broad macroconidia in pionnotes or in one or two ochraceous-salmon (Ridgway) sporodochia 1-2 mm. in diameter; shape curved, pedicellate with the terminal cell curved and somewhat constricted at the end, typical of the group *Elegans*; mostly 3-septate, though a few 4- and 5-septate spores also occur.

	Average
Dimensions: 3-septate, 25-44 × 3-4.5 μ	37 × 3.75 μ

*Chlamydospores*: terminal and intercalary, produced in the medium and in the aerial mycelium, singly or in chains, those in the medium

sometimes blue in the mass; usually abundant in culture, but only occasionally present in the bulb; in old sporodochia formed from the segments of the macroconidia.

Average  
Dimensions:  $7-14 \times 6-13 \mu$   $8.5 \times 9.5 \mu$

*Strain B* differs from strain A in having somewhat broader macroconidia (dimensions of 3-septate spores,  $21-45 \times 4-4.7 \mu$ , Av.  $35 \times 4.5 \mu$ ); in the abundant formation of dark blue sclerotia on starchy media, and in the frequent presence of a blue stroma. In these characters strain B approaches *F. oxysporum*.

(c) *Identity of the Fusarium.*

From the presence of numerous microconidia, terminal and intercalary chlamydospores, and from the shape of the macroconidia the *Fusarium* is definitely placed in the section *Elegans* of that genus.

The description of *F. bulbigenum* given by the original authors Cooke and Massee (4) is too meagre to allow one to decide whether or not the bulb-rot organism belongs to that species. Accordingly three separate isolations of each strain were sent to Dr Wollenweber for determination, and all six were considered by him to be *F. bulbigenum* Cke. and Mass. He did not regard as important the *oxysporum*-like appearance of strain B but drew attention to the resemblances between it and *F. bulbigenum* var. *niveum* (Erw. Sm.) Wr. (Wollenweber (26)).

(d) *Varieties of Narcissus from which F. bulbigenum has been isolated.*

Isolations of *F. bulbigenum* were made from 116 bulbs, belonging to the following 21 varieties:

*Trumpet daffodils.* Emperor, Golden Sand, Henry Irving, Glory of Leiden, Golden Spur, Van Waveren's Giant, Loveliness, Peter Barr, Madame de Graaf, Empress, Grandis, Horsfieldii, Victoria, Weardale Perfection.

*Incomparabilis.* White Colossus.

*Barrii.* Conspicuous.

*Leedsii.* Chamois, St Sennens.

*Cyclamineus.* Cyclamineus.

*Poeticus.* Ornatus.

*Double Trumpet.* Cernuus plenus.

Three undetermined varieties.

In connection with the possibility of the existence of varietal resistance to the disease it may be noted that the *Fusarium* has not yet

been isolated from varieties belonging to the groups *Triandrus*, *Jonquil* and *Tazetta*, and to judge from the lack of references to these groups in the literature it is evident that they are not as a rule attacked.

The place of origin of the bulbs from which isolations were made could not always be determined, but infected stocks have been obtained both of English grown and Dutch grown bulbs.

(ii) *Fusarium moniliforme* Sheldon.

Two strains of this organism submitted to Dr Wollenweber were identified by him as variety *subglutinans* Wr. and Reink.

(a) *Cultural features.*

*Microconidia*: 0-1-septate, abundant in pionnotes, or in the aerial mycelium, formed in false heads or chains according to the humidity of the culture, as was shown by use of the slide-culture method of Vernon (17).

*Macroconidia*: 3-5(-7)-septate in salmon-pink sticky pionnotes or sporodochia, long, narrow, pedicellate, straight, of even diameter except at extreme ends.

Dimensions: 0-septate		Average
1.	4-14 × 2-3 $\mu$	8 × 2.5 $\mu$
1.	10-24 × 3 $\mu$	15 × 3 $\mu$
3.	30-58 × 3-4 $\mu$	43 × 3.25 $\mu$
4.	34-62 × 3-4 $\mu$	48 × 3.5 $\mu$
5.	38-70 × 3-4 $\mu$	54 × 3.5 $\mu$
6.	41-73 × 3-4 $\mu$	59 × 3.75 $\mu$
7.	61 × 4 $\mu$	

*Chlamydospores* and *sclerotia* not observed in culture, but a dark blue discoloration of the medium occasionally present locally.

*Optimum growth temperature* on Brown's synthetic potato medium about 20° C.

(b) *Occurrence of the fungus.*

Though isolated from twelve varieties this species was not obtained from the interior of any bulb showing typical symptoms of *Fusarium* rot, but it has been found in eelworm-infected bulbs of several varieties at the Chelsea Physic Garden and in Lincolnshire. *F. moniliforme* is also common on the outer brown papery scales of healthy Narcissus bulbs where it forms thin layers of white or pinkish mycelium (Plate XXVII, fig. 5). Such bulbs, however, do not decay. It has also been isolated from a blind flower stalk, from the leaf tips of a bulb grown in a pot in the laboratory, and from lesions on the underground parts of leaves in which the presence of *Tylenchus dipsaci* could not be detected.



Whole-bulb and single-scale inoculations with three strains of this fungus into several varieties of bulb have so far given negative results. It is suggested that *F. moniliforme* or its varieties may be the organism described by Massee as attacking the Narcissus leaf, and as producing "secondary spores" or microconidia in chains. If this interpretation is correct Massee's disease of Narcissus would appear to consist not only of eelworm in the leaves and of a chlamydospore-producing *Fusarium* in the bulb, but also of *F. moniliforme* on the leaves. This interpretation would agree with Westerdijk's observation<sup>(24)</sup> that *Fusarium*, when it does occur on the leaves, does not produce chlamydospores. It may be that the *Fusarium* found by Welsford<sup>(22)</sup> on Narcissus, and stated by her to be frequently present on the surface of healthy bulbs, was also *F. moniliforme* and not *F. bulbigenum*. This would account for the conclusion drawn by that writer that the *Fusarium* associated with rotted Narcissus bulbs is not pathogenic. On the other hand the discrepancy between Welsford's and the writer's findings may be due to the rather unfortunate fact that none of the varieties of bulb selected by Welsford for inoculation with *Fusarium* belongs to the highly susceptible Trumpet groups, and one of them, *Incomparabilis* "Cynosure", appears to be definitely resistant, as will be shown later.

The present work indicates that while *F. moniliforme* appears commonly on Narcissus plants, it is not responsible for the bulb rot disease.

(iii) *Cylindrocarpon radicicola* Wr. (*Ramularia macrospora* Wr. non Fres.)

(a) *Cultural features.*

Agar culture white at first, becoming brown later owing to the production of brown terminal and intercalary chlamydospores, often in chains (av. dimensions,  $8 \times 15\mu$ ).

*Conidia* borne in a thin layer over the culture, or in small yellow sporodochia, cylindrical, straight or slightly curved, ends rounded with a papilla at the base.

Dimensions:		Average
0-septate	$3.5-14 \times 2.5-4.5\mu$	$8 \times 3.5\mu$
1. "	$12-20 \times 4.5-7.5\mu$	$22.5 \times 6.5\mu$
2-3. "	$30-35 \times 7-8\mu$	$32 \times 7.5\mu$

(b) *Occurrence of the fungus.*

This fungus has been isolated twenty-four times from bulbs, viz. from dead basal tissue of the previous season's growth, from dead root stumps embedded in living basal tissue (Plate XXVII, fig. 6), and from

the surface of healthy scales. In addition it has been isolated from the roots of specimens affected with root rot. The only inoculations into basal tissue so far made with this fungus have given negative results, so that at present it is impossible to assign to it a parasitic rôle.

### C. INOCULATIONS WITH *F. BULBIGINUM*.

Two sets of experiments were carried out in this connection. In one series whole bulbs, in the other individual scales were used. These experiments will be dealt with separately.

#### (i) *Inoculation of whole bulbs.*

##### (a) *Material and methods.*

For inoculations a culture isolated from a rotted bulb of the variety "Madame de Graaf" was selected as being typical of strain A, and was used in most of the experiments to be described in this section. In a few cases, however, the inoculum was a slightly more virulent culture of strain B, from a Dutch "Victoria" bulb. All cultures used in inoculations were freed from bacteria, and purified by the hyphal tip method.

The bulbs used for inoculation and for the controls were selected from healthy stocks, and before inoculation the surface was washed with 95 per cent. alcohol; in some cases as an additional precaution against contamination the bulbs were given a cold soak for three hours in 1 per cent. formalin solution, and carefully dried before inoculating.

Inoculations of unwounded bulbs were made by placing a portion of mycelium, or of agar medium bearing a culture of the *Fusarium*, on the tissue, and keeping the bulbs in a moist dish. Controls had a piece of sterile agar placed on the surface of the bulb. In other cases unwounded bulbs were dipped in spore suspensions.

In the earlier experiments wound inoculations were made by stabbing the tissue through a portion of agar culture, a method which made only a small initial wound. Controls were stabbed through a piece of sterile agar placed in a similar position to the inoculum on the surface of the bulb. Such inoculated bulbs were kept moist in order to prevent the inoculum from drying up, and this was found to lead to the growth of moulds on the surface of the bulbs. The method finally adopted to overcome this difficulty was to lift up a V-shaped flap of basal tissue, and to introduce a portion of mycelium. Whether the flap was sealed with wax, or left unsealed did not appear to affect the result of the experiment. Control bulbs were wounded similarly without the introduction of the fungus, except in one experiment where they were not wounded but lay

alongside the inoculated bulbs in the incubator. Wound inoculations were also made by placing mycelium on the cleavage surface left by pulling apart the components of double-nosed bulbs.

Inoculated bulbs were usually placed in an incubator at 20° to 23° C. Sometimes, however, laboratory temperatures, or incubators at 25° or 27° C. were employed. The experiment was usually allowed to run for three or four weeks, after which the bulbs were cut open and examined. Occasionally in experiments conducted at laboratory temperatures a longer period was allowed.

Reisolations were made from a proportion of the successful inoculations by methods similar to those adopted in making the original isolations.

(b) *The effect of wounding.*

With certain exceptions to be stated later no success has been obtained in infecting bulbs by placing the fungus on unwounded tissue, whether on the basal ring or on the fleshy scales. On the other hand, a high percentage of infection results when the fungus is inserted into a wound. This is illustrated in Table II which gives results of an experiment with two susceptible varieties.

Table II.

Variety	Proportion of inoculated bulbs infected	
	Wounded	Unwounded
Madame de Graaf	7/8	0/8
Golden Spur	4/4	0/19

Table III gives a summary of the results obtained by inoculating wounded bulbs of several varieties.

Table III.

Variety	No. inoculated	No. infected
Madame de Graaf	24	20
Barrii Conspicuus	21	20
Poeticus Ornatus	12	12
Emperor	72	13
Leedsii Bridesmaid	6	0
Golden Spur	5	4
Obvallaris	50	50
Horsfieldii	8	8
Henry Irving	9	8
Sundry varieties	15	7
Total	222	142

It is clear that certain of the varieties such as "Madame de Graaf," "Barrii Conspicuus," "Obvallaris," etc., give a high percentage of in-

fection, while the variety "Emperor" appears to be somewhat resistant.

Over and above differences in percentage infection as shown in Table III, there were noted differences in the amount of attack. Thus, while varieties "Barrii Conspicuus" and "Madame de Graaf" both gave a high percentage of successful inoculations, the subsequent rate of invasion of the tissue was much greater in "Madame de Graaf."

In the experiments summarised in Table III on inoculating wounded bulbs, eighty-nine control bulbs (usually wounded but not inoculated) were used, and only one of them became rotted.

The exceptions, which the writer considers to be only apparent, to the rule that infection does not take place in the absence of wounding were as follows:

In one experiment thirteen bulbs of "Golden Spur" were inoculated during a form of hot water treatment by immersing them in water with *Fusarium* spores at a temperature of 36° C. for four hours. Thirteen control bulbs were treated in the same way in water from which the *Fusarium* spores were absent. The bulbs were at first left damp in closed dishes, and after one week were dried. When examined after a further three weeks eleven out of the thirteen inoculated bulbs were more or less extensively rotted from the base upwards, and *Fusarium* was reisolated from the rotted tissue in every case. The controls were healthy except for two bulbs with insignificant basal lesions yielding *Penicillium* and bacteria only.

In another experiment thirty bulbs of "Henry Irving" had their bases dipped in a suspension of *Fusarium* spores, and were placed in a damp dish at 27° C. Examinations which were made from ten to eighteen days after inoculation showed that twenty-eight bulbs had become infected. Isolations made from five of these bulbs gave pure cultures of *F. bulbigenum*. Of ten control bulbs dipped in sterile water and kept under the same conditions, one became rotted with *Fusarium*, the other nine remained healthy.

In neither of these experiments was there any artificial wounding. Nevertheless there was considerable attack when spores of *Fusarium* were present. The discussion of these results will be more conveniently taken later, in the section dealing with the mode of entry of the fungus into the bulb.

The results of the experiments on inoculating whole bulbs showed that *F. bulbigenum* was capable of producing the rot usually associated in storage with that fungus. The organism was reisolated from

seventy-one out of eighty-three bulbs which had rotted after inoculation. One of these cultures was again tested by inoculating separate bulb scales and found to be pathogenic.

(c) *The effect of variety.*

This is illustrated in Table III. The results shown there are a compilation from a number of experiments in which the conditions were not identical throughout. Thus in some cases inoculations were made by stabbing through a piece of agar culture, while in others mycelium was introduced into a flap in the basal tissue. Again, the temperature of incubation varied from 15° to 25° C. That the same result is obtained under strictly comparable conditions is shown by the figures in Table IV, which refer to experiments carried out under uniform conditions of inoculation in a 20° incubator.

Table IV.

Variety	No. inoculated	No. infected
Madame de Graaf	10	9
Poeticus Ornatus	12	12
Emperor	15	5
Bridesmaid	6	0

(d) *The effect of temperature.*

The dependence of successful infection upon incubation of the bulbs at an adequate temperature is well shown by an experiment designed to test the effect of cool storage in reducing the percentage rotting of inoculated bulbs.

Two hundred bulbs of "Obvallaris" were inoculated by inserting mycelium of *F. bulbigenum* into an unsealed flap in the basal tissue. The bulbs were incubated at 27° C. for three days in order to give the infection an opportunity to establish itself. Fifty of the bulbs were left at this temperature, while the other 150 were removed to a cool store operated at 10°–12° C. Uninoculated control bulbs were placed with the inoculated ones at each temperature. Batches were removed from the cool store periodically for examination over a period of eleven weeks. Table V shows the effect of temperature upon the number of bulbs rotting.

Table V.

Treatment	Incubation temp. (° C.)	Time (weeks)	Rotted %
50 control, uninoculated	27	5	2 (bulb fly)
50 inoculated	27	4	100
50-control, uninoculated	10–12	11	0
150 inoculated	10–12	2½–11	0

Half of the bulbs removed from the low temperature store on each occasion were replaced in the 27° C. incubator to test whether infection was capable of being restarted, the remainder of the bulbs being used for a different purpose. The result of this treatment is shown in Table VI.

Table VI.

No. of bulbs	Weeks at 10°-12° C.	Rotted at 10°-12° C. %	Weeks (subsequent) at 27° C.	Rotted at 27° C. %
50	0	—	4	100
25	2½	0	4	72
25	6½	0	4	75
25	11	0	6	55

That the fungus remained alive over the period of low-temperature storage is shown by the fact that when returned to the higher temperature, from 55-75 per cent. of the bulbs rotted. On the other hand, the failure of some of them to decay when placed at 27° C. may indicate that the cool storage allows the host tissue to set up some kind of resistance. An examination of some of those bulbs in which *Fusarium* attack had been permanently checked showed that the region of the inoculum had been walled off by the formation of cork, together with the deposition of some gummy material in the cells.

(ii) *Inoculation of separate bulb scales.*

When whole bulbs were inoculated it was impossible to trace from time to time the progress of the fungus in the bulb tissues. Surface indications were found to be a very unreliable guide to the amount of rotted tissue present, and detection of the early stages of the rot appeared to present considerable difficulty even to experienced bulb-warehousemen. With the object of seeing at what rate the fungus attacked the tissue, single-bulb scales were inoculated with *Fusarium* in moist Petri dishes. It was hoped that this method would give information on the relative susceptibility of different varieties, and perhaps would also be of value in connection with control measures by enabling one on cutting open a rotted bulb to estimate roughly the date at which infection became active. Incidentally the method has a great advantage in economy of material, at least as regards exploratory work.

In these experiments sound bulbs were selected, rinsed in 95 per cent. alcohol, the nose, base and outer scale leaves removed with a scalpel and the bulb cut longitudinally once or twice. The fleshy scales so exposed were separated, and care was usually necessary to ensure that each scale retained both its inner and its outer epidermis. In this way six to twelve

scales or portions of scales could be obtained from each bulb. The scales were put into moist Petri dishes. At first wet filter papers were used to keep the atmosphere moist, but it was found that repeated opening of the dish for the addition of sterile water to replace loss by evaporation led to contamination with moulds, so the following method was adopted. Petri dishes, each containing a watch-glass, were sterilised in the oven, and when cool the bottom of the dish was poured with agar containing 1 per cent. mercuric chloride. The scales were then placed in the watch-glass. This method was found to keep the atmosphere moist over a long period, and materially to reduce contamination by *Penicillium*. A high degree of humidity was necessary as it was found that if the separated scales were allowed to dry they turned brown and soon died. The scales were finally inoculated on the freshly cut lower edge with a small portion of *F. bulbigenum* mycelium, with or without agar. The radial advance of the discoloured (chocolate-brown) area was measured at intervals as accurately as the sometimes indefinite margin of the discoloured area would allow.

The progress of invasion was tested in this way on the scales of twenty-two varieties. The results obtained for seven of these varieties are shown graphically in Text-fig. 1. Each point on the graph gives the average obtained from five to ten scales. It is clear that with different varieties there are great differences in the rate of invasion. In general, scale inoculations showed the progress of attack to vary from less than 0.2 mm. per day in somewhat resistant varieties as "Sunrise" and "Seagull," up to 2 and 3 mm. per day in some of the highly susceptible White Trumpets.

On the basis of these results the varieties tested can be grouped roughly as susceptible and resistant:

*Susceptible. Trumpet.* Emperor, Golden Spur, King Alfred, Maximus, Van Waveren's Giant, Alice Knights, Loveliness, Madame de Graaf, Peter Barr, Horsfieldii, Hymen, Victoria.

*Incomparabilis.* Lady Margaret Boscawen, White Colossus.

*Barrii.* Conspicuous.

*Leedsii.* Mrs Langtry.

*Poeticus.* Cylgad, Ornatus.

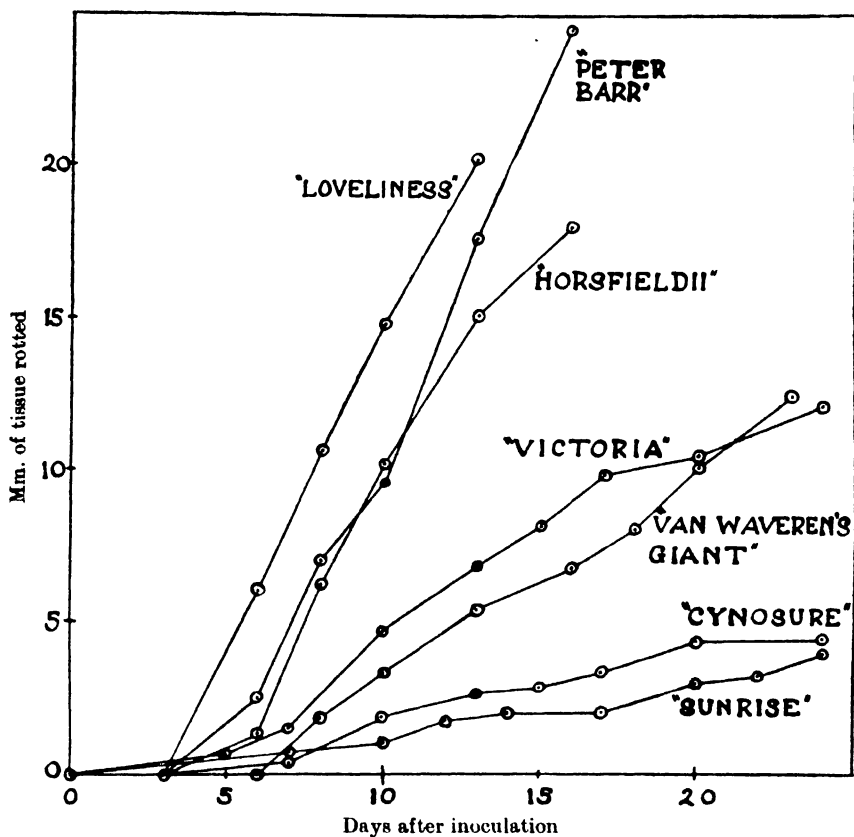
*Resistant. Incomparabilis.* Cynosure.

*Barrii.* Seagull, Sunrise.

*Leedsii.* Bridesmaid.

This grouping accords fairly well with the experience of growers, except that the varieties "Emperor" and "King Alfred" which are given

in the above list as susceptible do not appear to suffer from *Fusarium* in this country. On the other hand, to judge from information published by Weiss (20), the variety "Emperor" appears to be susceptible to a storage rot caused by an unspecified *Fusarium* in the United States. Although these results are of a preliminary nature, they indicate that the inocula-



Text-fig. 1. Illustrating different rates of invasion of seven varieties of *Narcissus* by *Fusarium bulbigenum*.

tion of single-bulb scales may be a promising method of determining the resistance of a new variety.

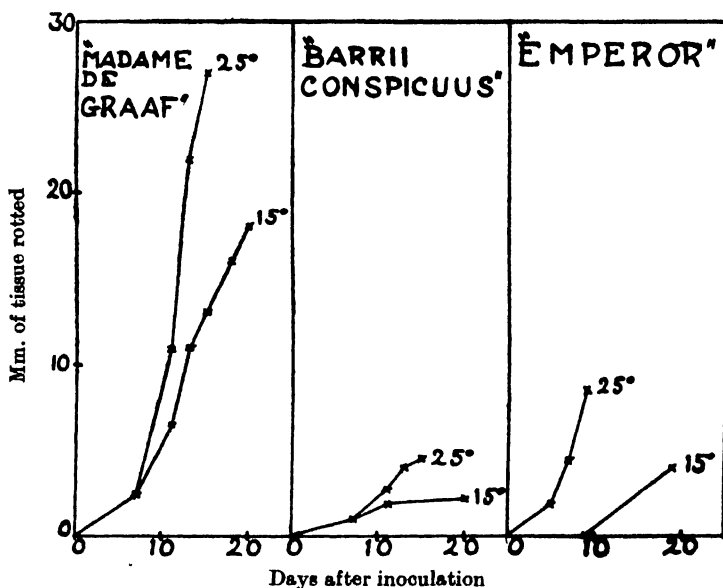
Preliminary experiments upon the effect of *temperature* on the rate of invasion were carried out by means of the scale inoculation method. An illustrative set of results is shown in Text-fig. 2.

It will be seen that while the three varieties tested are listed as susceptible, there are considerable differences between them in the rate of



invasion by the fungus. In all cases the effect of increased temperature in causing more vigorous attack is clearly shown.

The above data are based upon inoculations with a single strain of the organism. Eight other isolations of *F. bulbigenum* were tested by the single scale method and all proved to be pathogenic. Similar inoculations with two strains of *F. moniliforme*, with *Botrytis narcissicola*, *Trichoderma viride*, and with a strain of *F. fructigenum* from apple gave negative results.



Text-fig. 2. Showing effect of temperature on rate of invasion by *Fusarium bulbigenum*.

#### D. MODE OF ENTRY OF FUNGUS INTO BULB.

Some evidence has been brought forward to show that under ordinary conditions *F. bulbigenum* is unable to penetrate uninjured epidermal tissue of the bulb. The question, therefore, arises as to how the fungus is able to gain entrance to large numbers of bulbs under commercial conditions.

The mode of entry of the parasite was studied in a batch of 68 bulbs of the variety "White Colossus" which had been picked out by the foreman of a bulb warehouse simply as rotted bulbs, without any particular reference to *Fusarium*. It was interesting to find on examination that all these bulbs except one showed the typical *Fusarium* rot. Thirty of them

were too extensively rotted to yield any information as to the point at which attack began, but the remainder when cut open were found to be in a less advanced stage, and the position of the initial infection could be roughly determined as follows:

Rot progressing down through "nose" of bulb ... 9 cases

Rot progressing upwards from the base ... 28 cases

When infection began at the nose of the bulb, it was seen that it usually had begun in the old inflorescence axis. When, as in the majority of cases, infection was progressing from below, it appeared to be spreading from the neighbourhood of the basal ring, and especially from a cleavage surface where a secondary bulb had comparatively recently (presumably as a result of handling after lifting) been broken off a mother bulb.

In the observations just recorded, the percentage of bulbs showing rotting from the "nose" downwards was about 25 per cent. More extensive work indicated that this percentage, though small, is unduly high, as the following observations show.

In a stock of "Victoria" bulbs, naturally infected with *Fusarium* and sent over from Holland in September 1930 for experimental purposes, the problem of entry could be studied from another angle. A sample of 130 bulbs was divided into two lots, (a) "rounds" or single-nosed bulbs, and (b) double-nosed bulbs. When examined only 18 per cent. of the rounds were affected with *Fusarium* rot, while 61 per cent. of the doubles were beginning to decay. Rotting always appeared to progress from the base upwards, never down through the nose.

In the "doubles" the initial infection usually appeared in the commissure of basal tissue between two bulbs. It can easily be imagined that the tissue of this region is liable to considerable mechanical strain when the bulbs are handled or transported and cracks might arise here possibly giving access to the fungus. A particular case of this was observed in a diseased stock of "Spring Glory" in which the presence of sand grains had caused abrasions where the outer curved fleshy scale of a primary bulb touched its secondary. Streaks of rotted tissue were seen progressing down the scale towards the basal commissure. Further evidence of liability to injury in this region is the frequent perforation of the papery scale between the two bulbs and the occurrence of scablike corked-over patches on the opposed surfaces.

While thus there is considerable evidence that wounding through handling is of frequent occurrence, it is to be noted that infection can and does begin at any point round the base of the bulb.

The question now arises as to whether the roots furnish a point of entry to the fungus. There are two possibilities to be considered, (1) that the fungus enters the basal plate at the natural wound caused by the emergence of the root, and (2) that the fungus attacks the root surface and passes back into the basal plate. A number of experiments have been carried out in this connection, and, while not conclusive, and in need of repetition, they favour the first alternative.

In the experiment quoted on p. 489 "Henry Irving" bulbs were attacked after merely being dipped in a spore suspension and kept at 27° C. Some of the bulbs when examined after a few days clearly showed attack beginning where the tissue joining the base of double-nosed bulbs had become cracked, apparently through strains set up by the endogenous roots of each component growing across into the basal tissue of the other, and so presumably tending to push the two components apart. Others showed attack centring around points where developing roots had just penetrated the basal tissue to the exterior. In these cases the root itself appeared to resist attack longer than the surrounding basal tissue but succumbed in the end.

The stock of "Henry Irving" bulbs appeared to be on the point of emerging from their dormancy period, inasmuch as the root initials were very near the surface and were actually causing protrusions on the basal ring. It would be interesting in further work to examine how far there is a correlation between the degree of dormancy of the bulb and the incidence of *Fusarium* infection.

Similar attack at the point of emergence of roots in bulbs while out of the ground was found in three naturally infected bulbs of a stock of "Victoria," isolations from which yielded *F. bulbigenum*. Whether the attack through cracks in the commissure and around emerging roots is of common occurrence in storage and in the field can only be decided by further observations.

The susceptibility to attack of apparently unwounded bulbs which had been subjected to a hot-water treatment, as described on p. 489, cannot at the moment be explained. Further work should be directed to the question as to whether bulbs so treated, and especially when kept moist after treatment, are liable to show emergence of the roots. If that were so, the interpretation of the results along the above lines would be simple.

Attempts to inoculate the roots themselves have failed or have given inconclusive results. These experiments were carried out on bulbs the roots of which had been sprouted under laboratory conditions, and on bulbs growing in soil either in pots or in the field.

In two experiments fifty bulbs of "Emperor" were dipped in a spore suspension of the *Fusarium*, and the roots made to sprout by placing the bulbs in a moist chamber at 10–12° C. When the roots were 2–3 cm. long, by which time they were covered with a loose growth of the *Fusarium* mycelium, the bulbs were transferred to higher temperatures (15°, 20° and 25° C.). Mycelial growth was copious and the roots slowly turned brown and withered from the tip backwards, while those of uninoculated controls remained healthy. In none of the bulbs, however, did the fungus obviously penetrate basal tissue. In this respect therefore the results were negative, though it should be noted that the variety "Emperor" is not very susceptible to invasion. The mode of entry of the fungus into the roots in this experiment was not established.

In another experiment growing roots of five bulbs of the variety "Madame de Graaf" were allowed to dip into spore suspensions of *F. bulbigenum* for a period of one week at a temperature of 25° C. The plants were then removed to laboratory temperature and placed with the roots dipping into Knop's solution. This method failed to produce either discoloration of roots or rotting of basal tissue. The technique of this experiment may be criticised on the ground that the conditions of inoculation may not provide suitable conditions for the germination of the spores.

This objection does not apply to an experiment in which ten bulbs of "Poeticus Ornatus" were lifted in July, and after washing to remove soil, the roots were inoculated with portions of agar culture of the *Fusarium* in a moist dish. Both at laboratory temperature and at 27° C. the fungus failed to produce discoloration or rotting of the roots.

Inoculations were made on the roots of sixteen bulbs of "Golden Spur" each of which had been potted up separately on November 20th in the greenhouse. Below each bulb was inserted a length of glass tubing reaching to the surface of the soil where the end of the tube was plugged with cotton wool. On December 28th, January 25th, March 22nd and May 26th respectively, four pots were inoculated by pushing a portion of agar culture of *F. bulbigenum* down the glass tube into the neighbourhood of the roots. The end of the tube was again plugged. The bulbs were lifted on July 4th, and were stored. None of the inoculated bulbs or the controls appeared infected when lifted, nor did any rot during storage.

Various other experiments were designed to obtain infection by growing bulbs in proximity to the fungus. These experiments, the results of which have invariably been negative, may be summarised as follows:

(a) Eighty bulbs of "Golden Spur" grown in contact with or immediately above rotted bulb chips from which *Fusarium bulbigenum* had been isolated or above cultures of the fungus on potato slabs.

(b) Twenty bulbs of each of the highly susceptible varieties "Love-liness" and "Peter Barr" planted 1 inch above cultures of the fungus on potato.

(c) Bulbs of "Golden Spur" dipped in a spore suspension of the fungus before planting.

(d) Fifty bulbs of "Henry Irving" which had rotted with the *Fusarium* planted close to and in some cases in actual contact with twenty sound bulbs of "Golden Spur."

All the plants thus exposed to infection and control plants made good growth during the summer, and when lifted no *Fusarium* rot was noted.

The general conclusion drawn from these experiments is that the fungus does not attack living roots. There remains the possibility that it parasitises the moribund roots at the time they are dying off about midsummer, and passes from there to the bulb itself. Attention was paid to this point during the summer of 1930, and isolations were made from the bases of bulbs both before and after the normal time of lifting. *Cylindrocarpon radicicola* was several times found entering the bulb by way of root stumps dying back into the basal tissue, but *F. bulbigenum* was only found three times in this position. In view of this result, and of the failure of root inoculations it cannot be said that the present investigation provides any evidence that root infection takes place upon a wholesale scale from the soil, but the possibility must be borne in mind during future work.

#### E. CONTROL MEASURES.

Three types of possible control measures have so far been studied, with a view to reducing the amount of rotting caused by *F. bulbigenum*. These are (a) early planting, (b) attention to conditions of storage, and (c) treatment of stored bulbs with fungicides.

##### (i) *Time of planting.*

The view current among growers is that early planting of an affected stock materially reduces the amount of loss due to *Fusarium* rot. The evidence for this view is somewhat as follows. Stocks of bulbs, after lifting and cleaning, are sorted into various grades, the larger (*i.e.* those of flowering size) being stored for purposes of sale, and the smaller ones being planted as soon as convenient so as to grow further. The latter may

give a perfect stand of plants in the following spring while considerable loss may occur among the stored bulbs.

Two experiments were carried out in this connection, and these will now be described.

(a) *Experiment with variety "Poeticus Ornatus."*

In September 1929 a suspected stock of "Ornatus" was obtained and batches of fifty bulbs planted at approximately fortnightly intervals. The number of plants which were missing in the following spring from each batch is given in Table VII. Clearly in this case a great benefit was derived from early planting, but unfortunately this experiment proved not to be relevant to the present investigation, as the stock turned out to be infected with *Botrytis narcissicola*, and did not develop the typical *Fusarium* rot at all. Furthermore "Ornatus" is a variety in which the period of dormancy is short and not well defined, and perfectly sound bulbs of this variety are said to suffer for physiological reasons if not planted early.

Table VII.

Date of planting	No. of bulbs planted	No. rotted at time of planting	No. of plants missing in spring
September 17th	50	4	7
October 3rd	50	4	9
October 19th	50	8	29
November 9th	50	10	37

(b) *Experiment with variety "Victoria."*

Through the kindness of Dr G. H. Pethybridge and of Mr N. van Poeteren, a stock of *Narcissus* "Victoria" bulbs suspected of being affected with *Fusarium* was obtained for experimental purposes. So far as could be ascertained, the bulbs had not been treated with hot water against eelworm within the period of a year previous to dispatch. That this stock was, in fact, heavily infected with *F. bulbigenum* was apparent from the following observations. When received a few of the bulbs showed external signs of the disease, and during subsequent storage a substantial percentage decayed. Out of 160 isolations made from 60 of the rotted bulbs, 126 colonies appeared macroscopically to be typical of *F. bulbigenum*. Fifty-two of these were examined in detail and proved to be this fungus. These isolations were made from rotted bulbs at various times during the course of the experiments, so that it is clear that infection was present in the stock of bulbs from the beginning. Thirty-one of the decayed bulbs were examined for the presence of eelworms in the

tissues. No *Tylenchus* was found, and very few saprophytic eelworms were observed.

In the experiment to test the effect of date of planting upon the amount of rot, six lots of fifty bulbs each were planted at approximately fortnightly intervals from September 4th to November 8th. A number of the bulbs had rotted during storage, but these were planted with the sound ones, a record being kept of their number. The number of plants in each plot was counted in the following spring, poor shoots, even those consisting of a single leaf, being counted as one plant. This practice accounts for the fact that sometimes fewer missing plants were recorded than the number of apparently affected bulbs planted. The data are presented in Table VIII.

Table VIII.

Date of planting	No. infected when planted	No. plants missing	No. therefore rotted after planting
September 4th	4	13	9
September 21st	13	22	9
October 2nd	23	22	—
October 16th	15	16	1
October 31st	16	15	—
November 8th	24	23	—

The figures in the third column of Table VIII do not show any definite trend, *i.e.* the number of plants missing in the spring is not obviously related to the time of planting. Statistical comparison of the smallest and the largest figures in this column by a method of testing independence given by Fisher<sup>(9)</sup>, shows that the difference is not significant. On the other hand it appears from the last column of Table VIII that rotting has progressed in the soil in the case of the first two plantings, whereas in the last four there has been no further rotting of bulbs in the soil. This result has statistical significance when tested by the method cited above.

In marked contrast to the poor stand shown by infected bulbs, whether planted early or late, was the behaviour of control batches of "Emperor" and "Henry Irving" bulbs. These on examination in the bulb store showed no trace of *Fusarium* infection. They were planted in batches of fifty at various dates from the end of August to the end of October. A few bulbs of "Henry Irving" failed to come up in both the earlier and the later planting. This was due in part at least to a certain amount of fly infection. All the "Emperor" bulbs produced plants except one. The general conclusion is therefore that the seasonal conditions during the period of the experiment were such as to allow of practically a full

stand of plants from a healthy stock. The poor yield of the diseased stock can thus be definitely assigned to the presence of infection.

While the data given in Table VIII do not support the growers' view that early planting checks the disease, it will be well not to over-emphasise this result, as the number of bulbs used was not very large. The figures obtained indicate a considerable sampling error and the experiment should be repeated on a larger scale.

The view that the disease is checked by early planting is in general agreement with the writer's experience up to the present that no bulbs have been infected experimentally while in the soil. It is too early yet, however, to evaluate the significance of such negative results. On the other hand one might suggest that the growers' experience may be explained partly by the oncoming of other diseases (*e.g.* that due to *Botrytis narcissicola* illustrated in Table VII) and partly by a possible difference in susceptibility of the smaller bulbs planted from that of the larger flowering bulbs which are stored for purposes of sale. The liability to attack of large bulbs with offsets (see p. 495) should be remembered in this connection.

#### (ii) *Conditions of storage.*

The belief generally prevails that high summer temperatures are responsible for a high percentage decay of *Narcissus* bulbs of certain varieties. Jacob<sup>(12)</sup> and Westerdijk<sup>(23)</sup> both associate rotting due to *Fusarium* with these climatic conditions, and recently Griffiths<sup>(11)</sup> in U.S.A., without referring specifically to rot caused by *Fusarium*, speaks of "those atmospheric factors so detrimental on the Atlantic Coastal Plain." The same writer suggests that storage rot of *Narcissus* bulbs may be reduced by holding the bulbs during the hot weather at some temperature between 50° and 65° F.

Experiments on storage were therefore made from two points of view: (a) to test the effect of various conditions of temperature, humidity, etc., upon the incidence of rotting in a stock of bulbs affected with *Fusarium*, and (b) to find out how far, if at all, rotting of a healthy stock may be induced by adverse conditions of storage alone.

Bulbs were stored under conditions selected as follows:

- (1) 10°–12° C.: in wooden box with wire gauze sides in a cool store at Hay's Wharf, London Bridge.
- (2) *Air temperature, dry, London*: in open wooden tray in commercial bulb warehouse, Covent Garden.
- (3) *Air temperature, dry, Taplow*: in open wooden tray in an airy warehouse on a nursery, Taplow.



- (4) *Air temperature, dry, Slough*: in open wooden tray in airy room at Biological Field Station, Slough.
- (5) *Air temperature, moist*: in an inverted bell-jar, kept moist, with paper loosely tied over mouth of jar to ensure reasonable ventilation, at Slough.
- (6) 20° C., *dry*: in open cardboard box, in electrically controlled incubator, South Kensington.
- (7) 25° C., *dry*: as (6).
- (8) 25° C., *moist*: in unstoppered glass vessel over water in an electric incubator.
- (9) 30° C., *dry*: as (6).

(a) *Experiment with variety "Victoria."*

A stock of Dutch "Victoria" bulbs was divided into lots of fifty and stored under the conditions listed in Table IX. The period of storage ran from the beginning of September to the beginning of November, after which the bulbs were planted in beds freshly prepared by breaking up old meadow land in order to avoid any likelihood of eelworm infected soil interfering with the experiment. Planting was purposely delayed (November 7th and 8th) in order that storage under the various conditions should be carried on for as long as possible. The number of bulbs affected at the date of planting and the number of plants missing in the spring are shown in the following Table.

Table IX.

Conditions	No. infected on planting November 1930	No. missing April 1931
(1) 10°-12° C.	18	19
(2) Air temp. dry, London	24	23
(3) " " " Taplow	25	21
(4) " " " Slough	24	23
(5) " " " moist	28	28
(6) 20° C. dry	37	37
(7) 25° C. dry	40	39

In the spring bulbs from all treatments gave good plants, which appeared to be well rooted and which flowered.

The effect of maintaining the bulbs at warm temperature, such as those occurring commonly during the summer, over a considerable period is shown to be a substantial increase in the amount of rotting. The lowest figure is that of bulbs kept in cool store, and the figures for the tests at air temperature in London and in the country districts agree remarkably well. There is no suggestion therefore that the conditions of

storage at the London warehouse were inferior, as was at first considered possible.

The percentage of bulbs rotted (36 per cent.) in the cool store may be contrasted with the failure to produce infection of healthy bulbs at that temperature (Table V). One may reasonably postulate that a certain proportion of the "Victoria" bulbs in the experiment of Table IX were already infected at the time when the period of cool storage began. It is probable that the beneficial effects of cool storage would be more pronounced if the bulbs were so treated sooner after lifting.

(b) *Experiment with varieties "Henry Irving" and "Emperor."*

Under the same conditions as those outlined above, the experiment was repeated on sound stocks of "Henry Irving" and "Emperor" so that by comparison of the effects of the different treatments upon healthy bulbs in this experiment the effect of similar treatments upon the course of the disease in an infected stock could be the better judged. The experiment also served to show how far environmental conditions produce physiological disease in a presumably uninfected stock. It does not, however, form by any means an ideal control to the experiment with the infected Dutch "Victoria" bulbs, the most serious defects being that the two control stocks belonged to different varieties, were grown in different localities ("Henry Irving" in Devonshire, and "Emperor" in Lincolnshire) and were harvested and cured under possibly different conditions from the stock of "Victoria." However some of the tests applied to the stock of "Emperor" were severe, and might be expected to give positive results if unfavourable environmental conditions acting on dried bulbs were alone sufficient to produce decay.

"*Emperor.*" Batches of fifty bulbs of this variety were stored from August 20th to October 29th under the following sets of conditions: cool store (10°–12° C.); air temperature, dry; 20° dry; 25° dry; 25° moist; 30° dry.

All the bulbs appeared sound at the end of the treatment, even though those which had been kept moist at 25° C. were covered with a black slimy growth of bacteria and moulds. All were planted at the end of October and gave practically complete stands in the following spring.

It was interesting to note during the course of this experiment that cool storage accelerated root emergence whereas there was no trace of this in the bulbs stored at 20°–30° C., even in the case where the conditions were kept moist.

"Henry Irving." This variety, unlike "Emperor," is very susceptible

to *Fusarium* attack. In the particular stock used there was no trace of the latter disease, though in other respects the stock was inferior, being to a certain extent affected with root-plate rot and with bulb fly. The conditions of storage were of a similar type to those detailed above for the "Emperor" bulbs. The stand of plants in the following spring was approximately 90 per cent. for each batch, a lower figure than that obtained for the "Emperor" bulbs, but not unexpected in view of the inferior quality of the stock in the present case.

The effect of storing a healthy stock under various conditions may be summarised briefly. In spring, 1931, bulbs kept at 10°–12° C. during the previous summer months were somewhat ahead in development as compared with those kept at normal temperatures, and the flowers opened a few days earlier. While bulbs stored dry at 20°, 25° and 30° C., and in a saturated atmosphere at 25° C. all gave healthy well-rooted plants, the shoot appeared above ground from three to four weeks late, and the flowers opened one or two weeks later than those of control bulbs. The high temperatures had a pronounced effect upon the flowers, which were usually small, with perianth lobes often unequal in size, the trumpet nearly straight, and not expanded at the mouth. Leaf-mottling, as often seen after hot-water treatment, was not noted. Rotting or decay of the bulb tissues, or injury to the root initials, was not induced by any of the treatments, even two months' exposure to 25° C. in a saturated atmosphere with poor ventilation giving healthy plants.

These two experiments upon the storage of healthy stocks of dried bulbs under various conditions afford evidence that *Narcissus* bulbs can withstand temperatures warm enough over a period of two months to cause retardation of shoot and distortion or even blindness of the flower, and warm humid conditions which allow the copious development of bacteria and moulds, without suffering decay. The evidence presented is thus against the view that adverse storage of well-cured bulbs can cause rotting, and the deleterious effect believed to be exerted by warm temperatures upon the keeping of *Narcissus* bulbs must be due to the action of heat, not directly upon the bulb, but upon the complex "bulb plus fungus." That such a relation exists has already been indicated by the results of storing an infected stock at warm temperatures. It is suggested therefore that the effect of environmental conditions during storage is merely to aggravate or to check pre-existing infections.

That *narcissus* bulbs can withstand considerable exposure to adverse conditions is confirmed by a laboratory experiment in which fifteen "Ornatus" bulbs were set up under each of the following conditions:

(a) In closed vessel at laboratory temperature over alkaline pyrogallol (three weeks).

(b) Similar to (a) but at 30° C.

(c) At 30° C. but with free access of oxygen.

(d) At laboratory temperature but in an atmosphere containing 80 per cent. CO<sub>2</sub> (two weeks).

The bulbs kept under treatment (b) were found to be killed, with evolution of a peculiar cheesy smell. The same smell was just noticeable in the bulbs stored under condition (a) though they appeared healthy on the surface, and even when cut open. Some damage, however, had clearly taken place, as shown by the fact that out of four of these apparently sound bulbs planted, only two grew.

The bulbs under treatment (c) were perfectly normal and gave a complete stand of plants. Those under treatment (d) also appeared normal and gave a good though not a complete stand.

This experiment indicates that a moderately high temperature such as 30° C. is not in itself injurious so long as oxygen is freely available. High concentrations of carbon dioxide may be injurious, but it is probable that this factor is of subsidiary importance to scarcity of oxygen.

### (iii) *Treatment with fungicides.*

The common observation, that apparently healthy bulbs lying undisturbed in store may suddenly begin to rot, led to the hypothesis that in these bulbs the *Fusarium* had been lying dormant on the superficial tissues for some time, and that infection had been stimulated into activity during the period of storage by some change either in the bulb or in the external environment. If this is the case, it might be expected that surface sterilisation might lead to a reduction in the number of bulbs rotting.

Experiments along these lines were therefore carried out from three points of view, (a) to test the effect of surface sterilisation with fungicides in reducing the number of bulbs rotting in an infected stock, (b) to test the effect of various concentrations of these fungicides upon healthy bulbs, in order to get some indication of the treatment that could be safely applied without injury, and (c) to test the toxicity of various concentrations of fungicides to *Fusarium* spores.

#### (a) *Surface sterilisation of infected stock.*

It was decided to test the effect of surface sterilisation in combination with the hot-water treatment against eelworm on an infected stock of "Victoria" bulbs. Lots of fifty bulbs, heat treated with or without the

addition of fungicides to the water, were compared with bulbs untreated, but otherwise stored under identical conditions and planted at the same time. Treatments were carried out on September 11th and 12th by immersing the bulbs in water maintained at 43.5° C. and controlled to within 1° C. for a period of three hours from the time the required temperature was reached. The bulbs were then dried and stored in trays until November 7th, in order to find the number of bulbs which decayed during storage after each treatment. The results of this experiment are set out in Table X.

Table X.

Treatment	No. of bulbs affected when planted, 1930	No. of plants missing, 1931
Hot water only	33	33
Hot water with 1 % formalin	17	13
" " " 0.25 % Uspulun	20	22
" " " 0.5 % " "	20	18
Control, untreated	24	23

Dealing first with the bulbs which have been subjected to hot-water treatment, we note that the addition of the fungicide considerably reduces (by approximately one-half the number of bulbs which subsequently develop *Fusarium* disease. The difference between the formalin-treated and the Uspulun-treated bulbs is small but, if anything, in favour of formalin, especially on the basis of the results shown in the last column.

The hot-water treatment in itself appears to be harmful, as a comparison with the behaviour of the control (unheated) bulbs shows. This conclusion is in agreement with the work of Weiss (20). It is also borne out by the results of the experiment described on p. 489, in which unwounded bulbs of "Golden Spur" became infected after being heated in a suspension of spores. It should be noted however that *Fusarium* rot has been found in stocks which have not been subjected to the treatment for years, and there is no reason to suppose that the eelworm treatment is the only way in which the disease is initiated.

The results contained in Table X are the only ones so far available on the subject of fungicidal control of the disease in storage. The question of the safety of particular chemicals for use in this connection will be dealt with more fully below, but as regards the particular experiment under consideration neither formalin nor Uspulun produced any harmful effects. The foliage growth of the chemically treated bulbs in the following spring was excellent and there was no sign of root injury.

It should be remarked that the treatments outlined in Table X were given to bulbs some considerable time (probably six to eight weeks) after the usual date of lifting. During that interval of storage it is possible that *Fusarium* rot was progressing. There is a probability therefore that if treatment had been given at an earlier stage better control would have been obtained. So far as is known there is no need to combine the fungicidal treatment with the hot water bath against eelworm, and thus it is not impossible that an early steeping in cold fungicide would be effective in controlling *Fusarium* rot without involving any risk of damage to the flowers such as may be caused by the application of heat too soon after lifting.

The rational application of fungicidal treatments to bulbs will be based upon a knowledge of the distribution of the organism and of the sources of infection from which the bulbs become infected. The questions arising are:

(i) Is the organism present during the early part of the storage period on the surface of healthy bulbs which subsequently decay?

In this connection an experiment was carried out in which 200 isolations were made from the dead tissues present at the bases of fifty apparently healthy bulbs picked out of a batch in which a number of *Fusarium* rotted bulbs had already appeared. Three of these bulbs gave *F. bulbigenum* a number which is small seeing that, besides these three, fourteen other bulbs subsequently rotted with *Fusarium* during the autumn. Saprophytes were obtained in abundance. From time to time also in other tests *F. bulbigenum* has been isolated from the surface of healthy bulbs.

The observations so far made, which are to be regarded as preliminary, indicate that the parasite is not abundantly present in the isolations made from healthy bulbs which subsequently decay. Somewhat detailed isolations would be necessary to determine its full distribution over a stock of bulbs.

(ii) Do bulbs become contaminated while in storage from sacks, trays and warehouse fixtures or from spores blown from the surface of rotted bulbs?

That the spores of *F. bulbigenum* are capable under certain conditions of being blown about in the air was shown by the following experiment. With the help of a special apparatus, a current of dry filtered air was directed on to the surface of a culture placed in a large chamber. From another part of the vessel a current of air was withdrawn and passed over the surface of a sterile agar medium which could be changed periodically

and incubated to enable any spores blown over to germinate. Experiments with the apparatus showed that spores were not carried over by currents of air moving with a velocity of about 1 m.p.h., but air currents of something like 4 to 10 m.p.h. sometimes carried over *Fusarium* spores. The spores of *Aspergillus niger* were readily carried at this velocity. That the spores of the *Fusarium* do carry in the air is a practical possibility not to be ignored as bulbs are frequently to be found in the store-bearing cushions of mycelium-forming microconidia.

Only one experiment has so far been performed to determine whether contact with rotted bulbs is liable to produce rotting of sound ones. Twelve sound bulbs of the susceptible White Trumpet variety "Love-liness" were placed in a paper bag mixed up with a number of completely rotted bulbs of "Peter Barr" bearing external mycelium of the *Fusarium*. The bulbs remained in contact from August 27th to December 3rd and during this period none of the healthy bulbs became rotted.

Isolations made from boxes, structural woodwork and walls of a bulb warehouse in which the disease was common yielded negative results.

The inference to be drawn provisionally from these experiments is that bulbs do not become contaminated after being placed in the warehouse, that is, so far as decaying during that particular storage season is concerned. The possibility is not excluded that a bulb entirely free of the parasite may be contaminated while in store and in consequence undergo decay in the following year. No data on this point are as yet available.

The most probable theory at the moment is that the bulbs, or at least a proportion of them, are already contaminated when lifted, the potentially active parasite being established in the dead tissue at the base of the bulb. Under conditions not as yet fully specified the fungus proceeds to attack the neighbouring living tissue. Any further contamination which may occur after the bulbs have become air-dried in storage would appear to be of no significance, at least as regards that particular period of storage.

(b) *Effect of fungicides upon sound bulbs.*

The most promising control method as yet available is the steeping of the bulbs in a fungicidal solution. One would anticipate that the best results would be obtained if treatment were adopted immediately the bulbs were placed in storage. While there are strong indications that the incorporation of a fungicide in the water of the hot bath for eelworm is of value, this hot-water treatment cannot be recommended for bulbs at the beginning of the storage period on account of the risk of injury to the

flowers of the year following. Some kind of cold steep may thus prove to be the most useful method.

As a first step towards working out a control method of this type, experiments have been begun with the object of finding what concentrations of certain fungicides are injurious to the bulbs themselves. The bulbs are planted after treatment and their subsequent behaviour noted. Table XI gives a record of the treatments tested in 1930-1. A similarly extensive series, in which the treatment was begun earlier in the season and which includes a large number of cold-steep tests, is in progress.

Table XI.

Date of treatment	Variety	Treatment	Fungicide	No. treated	No. of plants
September 11th	Henry Irving	Untreated	None	50	47
		Hot water	None	50	49
		"	Formalin 1 %	50	46
October 15th	Henry Irving	Dry dusted	Ceresan	25	25
		Untreated	None	25	25
November 20th	Golden Spur	Untreated	None	20	20
		Hot water	None	20	19
		"	Uspulun 0.25 %	20	20
		"	Formalin 0.1 %	20	20
		"	Formalin 0.5 %	20	19
		"	Formalin 1.0 %	20	17
		"	Formalin 5.0 %	20	15
November 20th	Evangeline	Untreated	None	20	17
		Hot water	None	20	15
		"	Uspulun 0.25 %	20	17
		"	Formalin 0.1 %	20	16
		"	Formalin 0.5 %	20	17
		"	Formalin 1.0 %	20	13
November 27th	Golden Spur	Untreated	None	50	50
		Dry dusted	Tillantin R	48	48
January 24th	Peter Barr	Hot water	None	3	3
		"	CuSO <sub>4</sub> 0.1 %	4	4
		"	CuSO <sub>4</sub> 0.5 %	4	4
		"	CuSO <sub>4</sub> 1.0 %	4	4
		"	HgCl <sub>2</sub> 0.01 %	3	3
February 3rd	Loveliness	"	HgCl <sub>2</sub> 0.1 %	3	3
		Cold steep, 2½ hours	None	14	13
		" "	Uspulun 0.25 %	14	14

Indications of injury by 1 and 5 per cent. formalin were shown by the bulbs of "Golden Spur" and "Evangeline" treated on November 20th. Both these stocks were at the end of their period of dormancy, as was shown by the presence of projecting root "buds" on the basal ring.

The promising results obtained with formalin in the treatment of an affected stock, together with its comparative cheapness, and its harmlessness to human beings, suggest that the use of this substance for



sterilising the surface of suspected bulbs will be worth investigating in greater detail.

(c) *Toxicity of fungicides to Fusarium spores.*

Weiss (20, 21) has suggested that the *Fusarium* spores may be disseminated, and the spread of the disease from infected to healthy bulbs assisted by the hot-water treatment, and has made recommendations for the addition of weak solutions of fungicides to the water of the eelworm treatment tank to kill floating spores. In following up this suggestion it was necessary to find the minimum concentration of certain fungicides which are toxic to the spores of *F. bulbigenum* when immersed in the solutions under conditions approximating to those of hot-water treatment.

It was realised that the method of determining toxic concentration by inoculating nutrient media in which the fungicidal substance is made up in various concentrations, and taking the lowest concentrations at which no growth was recorded, would not meet the case. During the hot-water treatment spores washed off the surface of an infected bulb would be immersed in the solution for a period of three hours only, and when the bulbs were dried some of the spores might adhere to their surface. It was therefore considered advisable to expose the spores to the action of the fungicide for three hours at the required temperature, and then to place them on a nutrient medium free from the toxic substance, as these conditions would more nearly reproduce the conditions of the hot-water treatment.

The method adopted was as follows: A series of concentrations of the fungicide were placed in sterile plugged tubes and, together with control tubes containing sterile water, were immersed in the hot-water bath. A piece of aerial mycelium or a suspension of spores from a culture bearing both conidia and chlamydo-spores was added to the solution and shaken to mix it thoroughly. After being left in the water bath for two and a half or three hours the tube was again shaken and a loopful of the liquid was taken out and spread over the surface of a slant of nutrient agar, or was placed in a tube of liquid medium (potato extract) to allow any germinable spores to grow. The cultures were subsequently examined to determine what concentrations were toxic. It was found necessary to leave the tubes for as long as ten days before the final examination, as in some of the higher concentrations growth might not become apparent until after some days. Whether this phenomenon was due to an inhibiting action of the fungicide upon the germination of the spores, or to greater

resistance to the fungicides on the part of the chlamydospores combined with an ordinarily slower germination of these structures was not investigated. The minimum toxic concentrations given in Table XII were those in which no development occurred after ten days. It is conceivable that lower concentrations, *i.e.* those which prevented germination during the first two or three days while the bulbs are drying might serve in practice to prevent the spread of the disease.

Table XII.

*Concentrations toxic to spores of F. bulbigenum in two and a half hours at 43° C.*

Fungicide	Toxic %	Not toxic %
Formalin (40 % HCHO)	0.03	0.01
Mercuric chloride	0.005	0.001 (?)
Uspulun	0.01	0.005
Ceresan	0.01	0.005

A number of control tubes in which spores were suspended in pure water and put through the eelworm treatment showed free germination.

Similar experiments carried out with solutions at room temperature gave toxic concentrations as follows:

Formalin between 1.25 and 0.25 per cent.

Mercuric chloride between 0.01 and 0.005 per cent.

A considerable number of commercial stocks were hot-water treated during 1930, with the addition of approximately 0.125 per cent. formalin to the water ("Steriform" 1 pint to 100 gallons). No controls were arranged, so that conclusions as to the result of the treatment could not be drawn with the confidence desired. But during the following spring all the treated stocks grew healthily, and showed no sign of disease, or of injury to roots, foliage or flowers.

#### IV. SUMMARY.

1. A brief review is given of diseases affecting the underground parts of Narcissus plants, with especial reference to the bulb rot associated with *F. bulbigenum* Cke. and Mass., the symptoms of which are described both in stored bulbs and in the field.

2. In isolations made from stored bulbs *F. bulbigenum* was found most frequently. Two strains of this organism are briefly described. Two other organisms of frequent occurrence in Narcissus bulbs, *F. moniliforme* and *Cylindrocarpon radicola*, are also described.

3. Inoculations into healthy bulb tissues with strains of *F. bulbigenum* were found to produce the typical symptoms of the storage rot, and the fungus was reisolated without difficulty. In general, wounding of the tissue was necessary for infection to take place. In a few cases where infection was obtained without artificial wounding, naturally produced wounds are suspected. Inoculations of roots failed to produce the disease.

Inoculations with *F. moniliforme* and *Cylindrocarpon radiculicola*, which are frequently found on diseased bulbs, failed to give infection, and it is concluded that *F. bulbigenum* is the causal agent of the bulb rot.

4. There is considerable variation in the resistance of different varieties to the fungus.

5. *F. bulbigenum* was occasionally found entering *via* the nose of the bulb, but usually it attacked from the base upwards. A very frequent point of attack was at the junction of the two components of a double-nosed bulb. So far no clear evidence was obtained of the fungus entering the base of the bulb from infected soil through the dying-back roots, as stated by Weiss to be the case in America.

6. High temperature and high humidity during storage were found to aggravate the disease in an infected stock, but in healthy stocks these same factors did not harm the bulbs or reproduce the disease.

7. The beneficial effect claimed for early planting of stocks as a means of checking the disease has not been substantiated. In the light of the figures obtained, the question must be considered to be open.

8. Support was found for the view put forward by Weiss that the disease may be spread during the hot-water treatment against eelworm.

9. The surface sterilisation of bulbs with fungicides appears to offer promise as a method for reducing the amount of rotting during storage. Experiments are described in which tests were made of the effect of various concentrations of fungicides on the health of the bulbs themselves and on the germination of *F. bulbigenum* spores.

This investigation was carried out in the Department of Plant Pathology, Imperial College of Science and Technology, South Kensington, and at the Biological Field Station of the College at Slough, Bucks. In addition to acknowledgments made in the text, thanks are due to Prof. Munro, Director of the Field Station, for the facilities afforded there; to Dr G. H. Pethybridge and Mr W. C. Moore, of the Plant Pathological Laboratory, Harpenden, for much assistance in the way of material and references to literature; to Messrs G. R. Barr and H. R. Barr for many facilities for study at their Taplow Nursery and Covent

Garden Warehouse; to Messrs the Proprietors of Hay's Wharf for cold-store accommodation, and finally to Prof. W. Brown for his continual interest and assistance throughout all stages of the investigation.

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#### EXPLANATION OF PLATE XXVII.

- Fig. 1. L.S. bulb of "Peter Barr" showing typical spread of *Fusarium* rot from the base up the scales.
- Fig. 2. Surface view of bulb of "Victoria," showing discoloration spreading up outer fleshy scales, and presence of white mycelium in region of basal ring.
- Fig. 3. L.S. bulb of "Peter Barr" dug in April, showing that disease may develop before time of lifting.
- Fig. 4. T.S. bulbs in similar condition to that shown in Fig. 3.
- Fig. 5. Surface view of bulbs of "Henry Irving" showing areas of pinkish white mycelium of *F. moniliforme* Sheld.
- Fig. 6. Bulb of "Van Waveren's Giant" with basal cushion cut across and showing infection by *Cylindrocarpon radicola* centring around root bases.

(Received December 10th, 1931.)



Fig. 1.



Fig. 2.



Fig. 3.



Fig. 4.



Fig. 5.

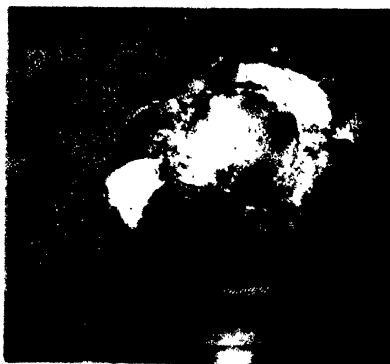


Fig. 6.



## ACTINOMYCES IN CACAO-BEANS

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THE occurrence of moulds in cacao beans is a constant source of trouble to cocoa and chocolate manufacturers. Apart from the hygienic undesirability of using moulded produce in the preparation of human food, the presence of moulds in cacao is economically deleterious because of the flavour they impart, the increased acidity produced in the valuable by-product cocoa-butter, and the mechanical loss due to the disintegration of the cotyledons.

A great many fungi have been isolated from the interior of cacao beans. These organisms vary considerably in frequency, intensity of infection, in their reaction upon their substratum, and in their taxonomic position. Usually no discrimination is made in the kind and effect of the mould present by purchasers of cacao. Cacao containing mould of any kind is undesirable, and packets in which is found a higher percentage of moulded beans than is permitted by trade regulations are subjected to arbitration and compensation. Such moulds are always plainly evident to the untrained eye, and very commonly consist of members of the *Aspergillus glaucus* group.

The mycological troubles of the cocoa manufacturers are not, however, confined to growths that are at once obvious to the unaided eye. For some years the produce of certain ports of South America has, it is said, been affected by an objectionable, musty odour which cannot be eradicated by the ordinary processes of manufacture. In 1930 a sample of similarly affected cacao imported to Amsterdam from Nigeria was sent to the Imperial College Stored Products Research Laboratory with a request that the cause of the smell be ascertained in order to frame regulations permitting buyers to refuse parcels containing beans affected in this manner.

Three *Actinomyces* and several other organisms were isolated from the Nigerian cacao, most of which had not before been found in cacao from that country. Unfortunately no data were available as to the treatment to which the cacao had been subjected, so that it is not possible to account for the occurrence of the unusual flora.



Enquiries confirmed the opinion that *Actinomyces* were not as yet a common defect of West African cacao. Isolations on several media clearly indicated that the pungent musty odour was due to the *Actinomyces*, and not to the other moulds, present. A similar result had been obtained by Ciferri<sup>(1)</sup>, who found that the frequent mustiness of cacao produced in the Dominican Republic was associated with the presence of five or more forms of *Actinomyces*, the commonest of which he considered to be a new variety of *A. albus*.

In the case of the Nigerian cacao the percentage of beans affected by *Actinomyces* was not high and so far as can be ascertained, the defect of mustiness is not common in cacao produced in West Africa, but for economic reasons and because of the importance of combating a possible menace to the industry at its earliest possible stage, it seemed desirable to attempt the identification of the organism concerned. To this end the Lister Institute was approached, and cultures were submitted to Dr Selman A. Waksman of New Jersey, who very kindly undertook an investigation of their morphology and physiology, and has also been good enough to provide the following descriptions of the organisms.

*Actinomyces cacaoi* WAKSMAN, NOV. SPEC.

*Strain I* (203 C).

*Cultural characteristics.* The organism produces on Czapek's and dextrose agar thin yellowish growth, later turning reddish brown, no soluble pigment; light grey to mouse-grey aerial mycelium, with white edge; typical odour of actinomyces. Nutrient agar: brown-coloured growth covered with tiny patches of ivory-coloured aerial mycelium. Flocculent growth on gelatin, no aerial mycelium; rapid liquefaction of gelatin without pigment formation. Potato plug: abundant brownish growth with white to mouse-grey aerial mycelium.

*Biochemical characteristics.* Strong proteolytic enzymes acting on casein and gelatin; strong diastatic action, no sugar or dextrin left in 1 per cent. starch solution after a few days. Limited reduction of nitrate.

*Morphology.* Long aerial mycelium with considerable spiral formation; the spirals are long and open, not compact.

*Classification.* Organism belongs to group BI in Waksman's system (2).

*Strain II* (203 F).

*Cultural characteristics.* Folded, thin, cream-coloured growth on Czapek's and dextrose agar, developing deep into the medium. No aerial mycelium at first; later on the surface of growth which is drying up

thin powdery mycelium develops. Sharp, pungent actinomyces odour. Abundant greyish growth on nutrient agar, no aerial mycelium. Thin, flaky growth on gelatin, no aerial mycelium; medium liquefaction, no pigment. Folded, grey growth on potato plug, later becoming covered with thin, powdery, white aerial mycelium.

*Biochemical characteristics.* Medium proteolytic action. Medium diastatic action, dextrin and sugar left within a few days of growth on 1 per cent. starch solution. Strong nitrate reduction.

*Morphology.* Short aerial mycelium, much branched; short spirals with few curls.

*Classification.* Belongs to group BI 7 of Waksman (2).

*Strain III (203 H).*

*Cultural characteristics.* Cream-coloured growth on synthetic and organic media, with abundant chalky-white aerial mycelium. Abundant growth with yellowish green tinge on potato plug, with extensive white-yellowish aerial mycelium. Growth on gelatin in the form of small specks and flakes with a characteristic yellow-coloured aerial mycelium; gelatin rapidly liquefied, without pigment formation.

*Biochemical activities.* Active proteolytic enzymes and fairly active diastatic. 1 per cent. starch solution, all starch disappeared in ten to twelve days, with some dextrin and considerable sugar left. Active nitrate reduction.

*Morphology.* Long aerial mycelium, well branched; few spirals, and these are only partly curved with only rare complete circles.

*Classification.* Group BI of Waksman (2).

SUMMARY.

The presence of *Actinomyces* within cacao beans is discussed, and descriptions by Waksman of three strains of a new species—*Act. cacaoi*—are given.

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(Received April 20th, 1932.)

## THE CHLOROTIC DISEASE OF THE HOP. III

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## HISTORICAL.

IN 1930 we recorded in this *Journal*<sup>(1)</sup> a new virus disease of the hop received from Worcestershire in 1927, to which we gave the name "Chlorotic Disease." Experiments were recorded there, and in a second paper<sup>(2)</sup>, detailing the various methods by which the disease has been transmitted artificially. The present paper records the results of inoculations carried out in 1930 with the Chlorotic Disease to ascertain (1) which commercial varieties of hops are susceptible to this disease, and (2) what results are obtained when an individual which is a symptomless carrier of mosaic disease is inoculated.

As regards the further spread of the disease under natural conditions, we can state, from information received during 1931, that the disease is still persisting in the hop-yard (of the variety Fuggle) in Worcestershire from which it was originally reported in 1927. During the season of 1931 we received examples from hop-yards in two fresh localities in Worcestershire; in one case the variety was Fuggle, in the other, Early Bird. A fourth case, in the same county, has been reported to us, on good authority, on the Tutsham variety.

I. SUSCEPTIBILITY OF COMMERCIAL AND NEW VARIETIES OF HOPS  
TO THE CHLOROTIC DISEASE.*(a) When grafted with chlorotic scions.*

The scions used were taken from four chlorotic-affected plants, Ch. 11, Ch. 18, R. 3/8, R. 3/11, individuals of the variety Fuggle obtained from Worcestershire in 1927. The stocks used, of the variety Tutsham (four plants) and Cobbs (three plants) were obtained in 1930 from a hop grower in East Kent, and were grown in pots. In April 1930 one scion was grafted on each of the stock plants; the scions lived and grew, with the exception of one on a Tutsham  $\frac{(30)}{8}$  and one on a Cobbs  $\frac{(30)}{7}$ . On the Tutsham, the scion, grafted on April 14th, was dying on May 15th; chlorosis was, however, transmitted to the stock (see below). On the Cobbs, the scion, grafted on April 14th, was dying on May 15th, and on June 3rd, 1930, the stock plant showed symptoms of mosaic disease. Of the two possibilities that the stock plant was already infected, or that the scion, as a symptomless carrier, communicated mosaic disease, the former appears the more probable, considering the early appearance of the symptoms. This mosaic-affected Cobbs plant was therefore discarded from the experiment.

During the season of 1930 no visible transmission of the chlorotic disease from scion to stock was observed, all the six remaining stock plants maintaining a healthy growth. In 1931 all six stock plants were diseased, the symptoms combining those of the mosaic and chlorotic diseases. Since this is the first record of the occurrence together in the same plant of these two diseases, a somewhat detailed description of the symptoms shown by each of the six stock plants is given below.

1. Tutsham  $\frac{(30)}{6}$ , Scion Ch. 11, June 4th. The plant had two stems, 30 in. and 24 in. high, both climbing. The taller stem had 15 nodes and chlorosis was visible on the leaves at the 9th–14th nodes. The laminae at the 10th and 11th nodes were distorted, being concave or convex in the central part. At the 12th and 13th nodes the laminae were not yet distorted and the chlorotic symptoms were no more than a suffused yellow colour in the pale green, particularly at the edges. The internodes were not shortened. One lateral (2 in. long) showed chlorotic leaves. The shorter stem had 16 nodes and chlorotic symptoms were present on the leaves at the 11th, 12th and 13th nodes. Convex and concave laminae occurred at the 12th and 13th nodes. There was no indication that mosaic disease was present except that the petioles were short and the laminae brittle. On July 3rd, however, both the tips were falling away from the string and the general appearance suggested mosaic disease. It was concluded that this plant was showing symptoms of both chlorosis and mosaic.

2. Tutsham  $\frac{(30)}{8}$ , Scion Ch. 11, June 4th. The plant had two stems, 22 in. and 21 in. high, and two short shoots,  $8\frac{1}{2}$  in. and  $3\frac{1}{2}$  in. The taller stem had 13 nodes; the leaves at the 5th to 12th nodes all showed symptoms of chlorosis varying from one or more chlorotic blotches on the lower leaves to large chlorotic areas combined with distortion of the lamina at the 7th to 11th nodes, where also the laminae were much reduced in size. This stem was of stunted appearance and, judging by the formation of laterals, had probably finished growth. The internodes were short and the tip not climbing. The shorter stem, also with 13 nodes, showed chlorosis on the laminae from the 4th node upwards, all the leaves from the 5th node upwards being also concave (boat-shaped) and very brittle or rigid. The general appearance of the plant was strongly suggestive of an attack by both mosaic and chlorosis, for in addition to the chlorotic areas and puckering of laminae, the stems were stunted and the leaves so brittle that they fell at a touch. Moreover, on the lower leaves of a short shoot ( $8\frac{1}{2}$  in. high) a faint mosaic mottling was present. On July 3rd, the appearance of the plant again made it possible to say with certainty that chlorosis was present; the only suspicious symptoms of mosaic disease at this date being the dwarf growth and falling away of the tips.

3. Tutsham  $\frac{(30)}{9}$ , Scion Ch. 18, June 4th. The plant had one brittle, diseased shoot only 1 in. high. Symptoms were indiscernible on the minute and very brittle leaves. By July 3rd this plant was dead.

4. Cobbs  $\frac{(30)}{23}$ , Scion R. 3/11, June 4th. The plant had two short shoots, 12 in. and 9 in. high. The longer had 16 nodes, the internodes were short and the laminae brittle and waved—characteristic of mosaic disease. The tip was also non-climbing. Laterals ( $2\frac{1}{2}$  in. long) were growing from the lower nodes and on one leaf of a lateral, two distinct chlorotic areas occurred. The shorter shoot (11 nodes) showed no symptoms of chlorosis but had an appearance of being affected with mosaic disease very similar

to that of the longer shoot. The impression was gained that the whole plant was suffering from mosaic disease and that the chlorotic symptoms on one leaf showed that that virus was also present.

5. Tutsham  $\frac{(30)}{24}$ , Scion R. 3/8, June 4th. The plant had two shoots,  $23\frac{1}{2}$  in. and 8 in. high, and some smaller shoots. The longer, with 16 nodes, showed down-curling and brittleness of the laminae (but no mottling) at the 7th to 9th nodes. At the 10th the laminae were down-curved and on each was a single area of suffused yellow near the margin of one lobe. At the 11th node both leaves were concave (boat-shaped) and a chlorotic blotch was present on one. The remaining leaves were small, with irregularly waved margin. The tip, with short internodes and non-climbing, was typical of mosaic disease. On one leaf of a lateral growing from a lower node, chlorotic markings occurred. On the 8 in. shoot, with 10 nodes, the internodes were short; the shoot was rigid, non-climbing, and mosaic mottling was visible in the upper leaves (from the 5th node upwards). While the general growth of the plant was more suggestive of the effects of mosaic than of chlorosis, the chlorotic disease was clearly discernible. On July 10th a further shoot (8 in. high) was present on this plant. It was free from any symptoms of mosaic and on one of the lowest leaves, definite chlorotic markings occurred near the margin.

6. Cobbs  $\frac{(30)}{25}$ , Scion R. 3/8, June 4th. This plant had one shoot,  $6\frac{1}{2}$  in. high, and two smaller shoots. The  $6\frac{1}{2}$  in. shoot had 13 nodes and at the 8th to 12th the laminae were of a suffused yellow-green colour. The stem was rigid, non-climbing, and with short internodes. The leaf symptoms were not typically those of mosaic disease; there was no mottling and no chlorotic areas were clearly defined. The two smaller shoots were weak but with no disease symptoms. On July 3rd the longest shoot was dead. The other two were dwarf and rigid, with small leaves. The appearance was suggestive of mosaic but mottling was not clearly enough defined to allow of any diagnosis.

The results of the above experiments show clearly that the chlorotic disease was transmitted by the scions to the stock plants, and that the symptoms were not shown until the following season. In every case when they appeared they were, with one doubtful exception, accompanied by symptoms of mosaic. The origin of the mosaic disease is probably to be ascribed to the chlorotic-affected Fuggle scions carrying the mosaic virus as well, rather than to infection of the stock plants previous to grafting<sup>1</sup>.

(b) *When used as scions on chlorotic-affected plants.*

1. *The variety Mathon used as scion.* Thirteen scions of Mathon were grafted in April 1930 on eight stocks. These stocks were chlorotic-affected individuals of the Fuggle variety obtained from Worcestershire in 1927. The scions were taken from two plants growing at Wye; the first (Ref. No. 120 a) was a clone plant taken in 1925 from the parent plant (R. 2/92) obtained under the name of Mathon from a grower in Kent

<sup>1</sup> Experiments have shown that the Fuggle variety is a potential symptomless carrier of mosaic and, under the conditions in the Experimental Hop-garden at Wye, it has become, with a few individual exceptions, an actual carrier. As regards the four chlorotic plants used, it is not known apart from the present evidence whether they carried the mosaic virus or not.

in 1914 and planted in the Wye Experimental Hop Garden. The parent plant remained vigorous and healthy until 1925, when it developed mosaic disease. The clone plant (Ref. No. 120 *a*) in 1928 showed doubtful symptoms of mosaic, but was vigorous and appeared healthy in 1929. In 1930, after scions had been taken from it, it showed (late in the season) undoubted mosaic symptoms in the leaves of several of the upper branches<sup>1</sup>. The second plant (Ref. No. R. 1/74 *a*) was obtained in 1921 from a grower in Worcestershire under the name of Mathon White; the plant remained healthy and vigorous until 1930, when, after scions had been taken, it showed severe mosaic symptoms late in the season.

The thirteen scions grafted showed the following symptoms during the season of 1930. The one scion (Ref. No. 120 *a*) on the stock plant Ch. 8 showed the symptoms of both chlorotic and mosaic disease. On July 8th this scion was 10 in. high, with a "blind" tip; there was clear evidence of the presence of mosaic disease in the mottling of the leaves. Two laterals developed; one, with four pairs of leaves, remained healthy, but the other showed chlorosis adjoining the veins of two of the three pairs of leaves present. A yellow band occurred on each side of the veins with a green ridge between. The appearance of the disease in the same season when it was grafted is noteworthy and is the second observed instance (see (1), p. 245).

Six scions (three from Ref. No. 120 *a* and three from R. 1/74 *a*) developed mosaic disease with no trace of chlorotic disease. The symptoms shown were the characteristic mosaic-mottling of the leaves, with the tip of the stem becoming thin and non-climbing. One scion (of 120 *a*) showed doubtful symptoms of mosaic. Five scions (two of 120 *a* and three of R. 1/74 *a*) remained healthy.

All of the scions when removed in the autumn of 1930 were too weak to be grown on, except one—the only and healthy scion of R. 1/74 *a* on the stock R. 3/8; this was potted up and remained healthy through 1931, growing to a height of 6 ft.

The results show that symptoms of chlorotic and mosaic disease may both become apparent in one and the same plant of the variety Mathon<sup>2</sup>.

The presence of mosaic disease in many of the scions is to be explained either by the fact that the stock plants were carrying this disease—since we know that Fuggle is a potential or actual symptomless "carrier," or that the scions used were already infected, since they were taken from a "tainted" stock.

2. *New seedling varieties used as scion.* On April 28th, 1931, a chlorotic plant (Ref. No. Ch. 11)<sup>3</sup> of the Fuggle variety, originally received from Worcestershire in 1927 and which had been grown in the open at Wye since 1928, was grafted with one scion

<sup>1</sup> As showing the susceptibility of this variety, it may be mentioned that all the other nine clone plants raised from R. 2/92 have become attacked by mosaic.

<sup>2</sup> As we have recorded ((1) 242), the grower in Worcestershire who first noticed the chlorotic disease stated that he had observed it occasionally on the Mathon variety.

<sup>3</sup> By May 29th, when the scions of GG. 45 and HH. 97 were 13 in. and 11 in. respectively, the chlorotic symptoms were strongly developed on Ch. 11—and were more pronounced than on any other of the twenty similarly diseased plants growing near by.

of each of two new seedling varieties, GG. 45 and HH. 97, raised at Wye from seeds of the Fuggle variety. The behaviour of these scions during the season was as follows:

GG. 45. On July 6th the scion had reached a height of 7 ft. The leaves at the first twelve nodes from the base were healthy; those at the 13th node (at 3 ft. 6 in. from the ground) showed a slight trace of chlorosis in the basal part of the lamina, and similar symptoms were present in the leaves at the 14th, 15th and 16th nodes. At the 17th node, one of the leaves, with rather more chlorosis, was distorted slightly. At the 18th and 19th nodes only a trace of chlorosis was visible on the laminae. On July 28th, the scion had reached a height of 10 ft. and showed exactly the same amount of chlorosis as on July 6th, the leaves at the 20th to the 28th nodes being healthy. On September 14th the scion was bearing well-formed hop cones. The chlorotic symptoms were still present, but the leaves and laterals from the 20th node upwards were healthy.

HH. 97. On July 6th, the scion was 3 ft. 6 in. high, with 18 nodes. On July 28th its height was 5 ft.; the tip of the bine was of pale colour and appeared to have ceased growth; by September 4th it was no higher. No trace of chlorotic disease appeared on this scion.

On November 16th, when the "hill" was "dressed," the bases of both the scions were removed, but they were too weak to be planted for observation in the following year.

The experiment shows that the seedling GG. 45 is susceptible to the chlorotic disease, and supplies the third known case of the appearance of the symptoms of the disease in the same season in which the virus was introduced. Although no symptoms of chlorosis appeared in the scion of HH. 97, it must not be assumed that this plant is resistant, for the symptoms of chlorosis commonly do not appear until the next season after grafting.

## II. INOCULATION OF SYMPTOMLESS MOSAIC "CARRIERS" WITH THE CHLOROTIC DISEASE.

### (a) *Chlorotic scions on mosaic "carrier" stocks.*

In April 1930 three mosaic-carrier plants, viz. D. 18, D. 8 and G. 15 were grafted with chlorotic scions. D. 18 and D. 8 are clone plants of a seedling variety, Ref. No. P. 13, raised at Wye in 1909, and later grown in some quantity at East Malling Research Station for testing on commercial lines. Here the variety has been subjected to attacks by the mosaic disease for many years<sup>1</sup>, and has proved to be completely resistant, never showing any symptoms. In 1927, experiments in which the two clone plants D. 18 and D. 8 were grafted with scions of Cobbs and Tutsham respectively, proved that both these plants were symptomless

<sup>1</sup> The evidence respecting the resistance of P. 13 during the years 1917-21 will be found in the *Fifth Report on the Trial of New Varieties of Hops*, 1921 (East Malling Research Station, 1922).

carriers of mosaic (3), p. 369). Similarly, the plant G. 15, which is a clone plant of the seedling variety M. 45, was proved also in 1927 to be a mosaic carrier (3), p. 368). In 1930 the mosaic-carrying property of all three was again tested: D. 18 was grafted with two scions of Cobbs on April 16th; on August 2nd one scion (3 ft. high) showed decided mosaic mottlings on the stem leaves, and also on the leaves of the laterals; the other scion grew to 16 ft. and remained healthy<sup>1</sup>. D. 8 was grafted on April 16th with one scion of Cobbs; this showed on August 2nd distinct mosaic mottlings on the leaves of every lateral; the scion grew to 15 ft. and bore cones by September. G. 15 was grafted on April 16th with one scion of Cobbs; the scion, which grew to only 1 ft., showed mosaic disease and was dead by August 2nd. Proof was thus obtained that these three stock plants were actual carriers in 1930.

As before stated, the plants D. 18, D. 8 and G. 15 were also grafted in 1930 with chlorotic scions. The latter were obtained from three individuals (Ch. 8, Ch. 16 and AA. 4) of the variety Fuggle obtained from Worcestershire in 1927. The behaviour of the scions and of the stock plants in 1930 was as follows:

D. 18. Two scions of Ch. 8 were grafted on April 14th. Both scions grew well, reaching by August 2nd heights of 15 ft. and 11 ft.; both showed chlorotic symptoms on the leaves throughout the season. On September 6th both chlorotic scions bore cones which were identified as those of the Fuggle variety.

On August 2nd the stock plant of D. 18 showed undoubted mosaic mottling on some leaves of the laterals. The presence of mosaic disease was confirmed on September 6th, when the bines were pulled down for examination; distinct mosaic mottling was present on the leaves of a few laterals of the bines at a distance of about 5 ft. from the ground and also on laterals near the top of the bines. Further, some of the cones of D. 18 were malformed, with fewer bracts and bracteoles than in the normal cone—this again being a symptom of mosaic disease. As noted below, mosaic disease was again apparent on D. 18 in 1931.

Two other cases of the appearance of mosaic in a symptomless carrier following grafting with chlorotic scions are given below. The present case is, however, very remarkable in that mosaic symptoms appeared in the same season that the grafting was done, and in not more than 110 days after the operation. The evidence appears to be conclusive that grafting with chlorotic scions was the cause, since the stock plant (together with the parent plant and a large progeny of clone plants) have been observed for many years to be actual or potential symptomless carriers of mosaic. Further experiments will be undertaken to confirm this point.

D. 8. Three scions of Ch. 16 were grafted on April 14th. All the scions grew well, and by August 2nd had reached heights of 14 ft., 13 ft. and 11 ft.; all the scions

<sup>1</sup> Other cases have been met with where mosaic-susceptible scions grafted on carriers have occasionally remained healthy. These will be discussed in a paper which will be published shortly.



exhibited marked symptoms of chlorotic disease. On September 6th the three scions bore hop cones. The stock plant of D. 8 showed no symptoms of mosaic during the season of 1930 and produced normal cones.

G. 15. This plant was grafted on April 29th—15 days later than D. 18 and D. 8 were grafted—and as no short scions were available, tips of more elongated stems of AA. 4 were used, and in order that they should fit the stock, it was found necessary to have them 7 or 8 in. long. By May 23rd one scion was alive and the other dead; on June 5th the one scion was still alive, but the tip was dead, and by July 4th this scion was dead also. No signs of any disease appeared on G. 15 during 1930.

In 1931 the three plants showed, on July 6th, the following symptoms: D. 18. Three stems ("bines") were trained up<sup>1</sup>; a short shoot and a "runner" were also present. *Bine 1*: about 9 ft. high. The lowest lateral, at about 5 ft. from the ground, was 18 in. long, with seven pairs of leaves; on the lowest four pairs of leaves, chlorotic disease was present, with brilliant coloration; at the next eight nodes of the bine the leaves showed chlorosis accompanied by the distortion characteristic of this disease<sup>2</sup>; at the 9th and 10th nodes traces of yellow coloration were present, and at the 11th and 12th nodes the leaves appeared healthy. Two other laterals, higher up the bine, showed chlorosis on the basal pairs of leaves. No symptoms of mosaic disease were noted on this bine. *Bine 2*: about 10 ft. 6 in. high. All the leaves of the main stem (except the top four pairs) showed chlorosis, usually in yellow splashes along the veins and along the margins, causing irregular lobing; sometimes the disease caused the leaves to be "domed." The laterals all the way up the bine showed chlorosis, at least on their lower leaves. No mosaic symptoms were noted on this bine. *Bine 3*: about 9 ft. high. The leaves at the various nodes showed the following symptoms: 1st node (at about 5 ft. from ground), one leaf showed mosaic mottling, but no chlorosis; the other leaf showed some mosaic mottling, and chlorosis was clearly evident on one lobe. 2nd node, both leaves showed mosaic mottling. 3rd node, one leaf with mosaic mottling and the other with decided chlorotic markings on the basal lobes. 4th node, both leaves showed faint mosaic mottlings. 5th node, one leaf showed a trace of chlorosis in the basal lobe. The leaves at the remaining nodes (6th to 11th) were green though rather distorted. No laterals longer than 1½ in. were present on this bine. The tip of the bine was not inclined to climb. It may be noted that all the stipules on all the three bines were brown and appeared to be symptomatic of some disease. The shoot present, coming from the "crown" of the rootstock, was only 2 in. long; it bore four leaves, all of which showed chlorotic markings. The "runner" shoot bore seven pairs of leaves, all healthy.

D. 8. Three bines had been trained up. *Bine 1*: 14 ft. high. Chlorosis was visible in the leaves of the upper part of the stem, and in the laterals. *Bine 2*: 16 ft. Marked chlorotic symptoms on all the leaves. *Bine 3*: 7 ft. Tips of the main stem and of the laterals not inclined to climb. Marked chlorotic symptoms on the leaves, accompanied by characteristic distortion. The leaves near the tips showed certain of the mosaic symptoms, being brittle and down-curved; the internodes also were short. Although no

<sup>1</sup> Following the usual cultural operations, all the stems not required for growing up the strings had been removed earlier. Further, the leaves of all the trained bines had been stripped off up to a height of 5 ft.

<sup>2</sup> See this *Journal*, 1930, xvii, Plate XXI, figs. 1, 2.

mosaic mottling was present, it was considered that the mosaic disease was appearing in this bine in addition to the chlorotic disease.

G. 15. Three bines (4 ft. 6 in., 3 ft. and 2 ft. 6 in.) were present, all of which showed characteristic symptoms of chlorosis, with large yellow areas on the leaves. No distortion of the lamina occurred, except the usual irregular margins. No mosaic symptoms were visible.

The results of the experiments showed that both the seedling varieties P. 13 (= D. 18, D. 8) and M. 45 (= G. 15) are susceptible to chlorotic disease. Further, that mosaic-carrier individuals of P. 13 when grafted with scions of the Fuggle variety affected with chlorosis may manifest symptoms of mosaic disease as well as those of chlorosis. Whether the chlorotic scions used carried the virus of mosaic disease as well (since the variety Fuggle is a potential carrier) is unknown. It must therefore be left to further experiments to decide whether a symptomless carrier of mosaic disease can be induced to exhibit mosaic symptoms by inoculation with the chlorotic virus alone, or whether a further dose of mosaic virus in combination with the chlorotic is necessary. In the case of M. 45 (G. 15), there was little union of scion and stock, and this may possibly have had some bearing on the fact that here no mosaic symptoms were shown. On the other hand, it is possible that the virus content of the scion used was different from that of the scions used in the two other experiments.

(b) *Mosaic-carrier scions on chlorotic-affected stocks.*

In April 1930 five scions from three individuals of two varieties of hops which had been proved to be mosaic-carriers were grafted on three chlorotic-affected individuals (Ch. 8, Ch. 12, Ch. 16) of the Fuggle variety obtained from Worcestershire in 1927. Of the five scions, three were obtained from the clone plants D. 18 and D. 8 of the seedling variety P. 13, and two from the American variety Red Vine Canada (Ref. No. R. 1/74). These clone plants of P. 13 were found in grafting experiments carried out in 1927 (3), p. 369) and in 1930 (see above, p. 523) to be carriers of mosaic disease. The plant of Red Vine Canada was shown in 1927 to be a carrier (3), pp. 364, 367).

The one scion of D. 18 and the two scions of D. 8, on the chlorotic stocks Ch. 8 and Ch. 16 respectively, grew during the season to a height varying from 2 to 3 ft. and remained healthy; the two scions of the Red Vine Canada on Ch. 12 grew to 10 ft. and 16 ft., and likewise remained healthy. The thickened bases of the scions ("strap cuts") were cut off, in March 1931, above the place of grafting, and were grown during 1931 as pot plants. All the plants formed shoots 2 to 4 ft. high and showed no sign of disease.

The results of the above experiment were therefore entirely negative. It is certainly surprising to find that the scions of D. 18 and D. 8 remained healthy, since when these stock plants were grafted with scions of Ch. 8 and Ch. 16, they developed chlorotic disease to a marked extent in the year after grafting (see above, pp. 524-5).

*(c) Chlorotic bud inserted on mosaic-carrier stock.*

As recorded in our last paper (2), p. 11), four plants (Ref. Nos. OK. 81, OK. 94, OK. 96, OR. 91) of the "Mid-European-Golding"—a variety believed to be identical with the Fuggle—were grafted in June 1929 each with a bud taken from the chlorotic plant Ch. 6, an individual of the Fuggle variety obtained from Worcestershire in 1927. All these four stock plants showed chlorosis in all their bines in the season of 1930. In 1931 all the four plants again showed chlorosis, and in one, OK. 94, mosaic symptoms developed in addition. In July this plant (OK. 94) was conspicuous on account of the shortness of the bines which had reached only 7 ft., as compared with 14 ft. to 16 ft. in the other three chlorotic plants. At this date, distinct mosaic mottling in the upper leaves of the main stem, and also in those of several shoots from the crown, was observable. On August 25th, the stems were only 8 ft. high, and showed chlorotic markings on the leaves of the main stem, up to the height of 4 ft. 6 in., while above that the leaves were curled and mosaic mottled. Mosaic mottling and chlorotic markings were not found on one and the same leaf. All the laterals were distinctly mosaic mottled and were not chlorotic. There could be no doubt whatever that this plant was exhibiting during the season of 1931 marked symptoms of mosaic disease as well as of chlorotic disease.

In order to ascertain whether the plant OK. 94 was a carrier of mosaic disease, a scion of a mosaic-susceptible variety (Cobbs) had been grafted on May 8th, 1931. By July 9th symptoms of mosaic disease began to appear on the leaves and the tip of the stem fell away from the string. By August 25th distinct mosaic mottling appeared on the leaves of the lateral shoots of the scion. It thus seems probable that OK. 94 at the time it was inoculated (in 1929) with a chlorotic bud was a symptomless carrier of mosaic disease; the case becomes therefore similar to that of the symptomless carrier P. 13, recorded above<sup>1</sup>.

<sup>1</sup> With the evidence now to hand, it seems very possible that the chlorotic plant V. 97—one of those originally sent from Worcester in 1927—which succumbed to mosaic disease during 1930, was a true Fuggle and not a mosaic-susceptible variety such as Mathon, which in our recent paper (2), p. 7) we suggested might have been the case. V. 97 had been grafted in 1929 with two scions from an individual (Ref. No. R. 1/44 a) of the Fuggle variety growing

With regard to the remaining plants, viz. the three "Mid-European Goldings" (OK. 96, OK. 81, OR. 91), the new seedling OK. 50 and the Fuggle plant OZ. 35, to all of which chlorotic disease was transmitted by budding (see (2), p. 11), but which showed no mosaic disease in 1931, the following evidence is available<sup>1</sup>.

The three Mid-European Goldings were grafted with mosaic-susceptible scions (Canterbury Golding); the two scions on OK. 96 both grew vigorously (12 ft., 10 ft.) and flowered and produced cones; the one scion on OK. 81 (grafted on April 14th) reached a height of 235 cm. by July 14th, and was then accidentally broken off at 140 cm. No definite symptoms of mosaic disease were observed on the leaves of the laterals which it then produced. The one scion on OR. 91 reached only 25 cm. and was dead on July 29th, without having shown any definite symptoms. It is evident that at least OK. 96 was different in 1931 from OK. 94 in not being a carrier of mosaic disease, since the latter plant was, as mentioned above, proved to be a mosaic carrier that year.

The new seedling OK. 50 was grafted in 1931 with a Canterbury Golding scion which reached 65 cm. and was attacked by Downy Mildew; it died on August 25th without giving any definite evidence.

The Fuggle plant OZ. 35 was grafted in 1930 with a scion of Canterbury Golding; the tip was attacked by Downy Mildew but the growth was continued by a lateral shoot which reached a height of 65 cm. and showed mosaic symptoms on the upper leaves.

It is not known whether the chlorotic plant Ch. 6, used for inoculating all the plants in this series, carried the virus of mosaic disease as well as that of the chlorotic disease.

#### SUMMARY.

1. Three further cases of the occurrence of the chlorotic disease of the hop, hitherto known from only one hop-yard (of the Fuggle variety) in Worcestershire, are recorded from hop-yards in the same county on the varieties Fuggle, Early Bird and Tutsham.

2. The chlorotic disease has been transmitted by grafting to the commercial varieties Tutsham, Cobbs and Mathon. With one doubtful

in the Experimental Hop-garden at Wye. There is no direct evidence as to whether this plant (R. 1/44 a) was an actual carrier of mosaic in 1929; individuals of Fuggles growing at Wye commonly become symptomless carriers of mosaic, and in an experiment in 1930 a scion of the Cobbs variety grafted on R. 1/44 a contracted mosaic disease. If, as seems possible, the scions of R. 1/44 a were mosaic carriers in 1929, this fact may have some bearing on the case. The other chlorotic plants, AA. 4 and V. 96 (see (1), p. 245) which up to date have shown no signs of mosaic disease, were each grafted with two scions from individuals of Fuggles growing in a commercial hop-garden which were probably not mosaic carriers.

<sup>1</sup> The five grafting experiments concerned (together with others to be published shortly) were carried out by Mr D. Mackenzie with the aid of a grant from the Ministry of Agriculture and Fisheries.

exception, all the affected plants showed as well the symptoms of mosaic disease.

3. The chlorotic disease has been transferred by grafting to three new seedling varieties (M. 45, p. 13 and GG. 45).

4. In three instances, individuals of symptomless mosaic carriers (of two varieties), to which chlorotic disease had been transmitted by grafting or budding, exhibited eventually marked symptoms of mosaic disease.

5. Two further cases are recorded of the appearance in scions of chlorotic symptoms in the same year in which the scions were grafted on the infected stocks.

#### REFERENCES.

- (1) SALMON, E. S. and WARE, W. M. (1930). The chlorotic disease of the hop. *Ann. App. Biol.* xvii, 241.
- (2) ——— (1932). *Idem*, II. *Ibid.* xix, 6.
- (3) MACKENZIE, D., SALMON, E. S., WARE, W. M. and WILLIAMS, S. (1929). The mosaic disease of the hop; grafting experiments, II. *Ibid.* xvi, 359.

(Received April 12th, 1932.)

#### APPENDIX.

While the above was in the press, two cases of a natural appearance of mosaic disease in plants, hitherto symptomless carriers, have come to notice. The first case was a further clone-plant (D 13) of the seedling variety P 13 mentioned on p. 522; the second was the German variety Prackenfels, obtained from Weihestephan in 1911. The latter plant showed symptoms of severe mosaic in the early summer of 1932 but produced later growth of healthy appearance; the plant of D 13 showed the general slight symptoms of a plant "sickening" for mosaic disease.

It seems well to record now these cases of the breaking down in nature of two among some hundreds of mosaic carriers in the same hop-garden—the first cases known to us—as their occurrence raises the question as to the validity of the explanation given above (p. 525) wherein the same phenomenon is attributed to the action of another virus artificially introduced into the plant. Experiments have been initiated in order to obtain further evidence, if possible, on this point.

# VIRUS DISEASES IN RELATION TO COMMERCIAL SEED POTATO PRODUCTION

BY T. WHITEHEAD AND J. F. CURRIE.

WITH A STUDY OF THE APHID POPULATION  
AT SELECTED FARMS

BY W. MALDWYN DAVIES.

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(With 1 Text-figure.)

## INTRODUCTION.

THE object of the present communication is to discuss the scientific principles underlying seed potato production in so far as they relate to the occurrence of virus diseases. It is recognised that other aspects, such as the proximity of suitable markets, the fluctuation of prices, methods of cultivation, and even the temperament of the growers may be of equal importance, but these considerations are reserved for a further paper.

It has been an integral part of the research of one of the writers since 1921 to ascertain under what conditions seed potatoes might be produced in North Wales. A survey extending over several years gave ample evidence of the existence of potentially good localities for the purpose, both in the health of the stocks which had long been maintained without change, as well as in the scarcity of aphides (1, 2). Properly replicated field trials showed that some of these old Welsh stocks gave as good a yield as seed from the north of Scotland (2). The practicability of maintaining stocks almost completely free from virus diseases was demonstrated, over a number of years, on the enclosed mountain land of the College Farm, near Bangor, although stocks of the same origin degenerated rapidly within the distance of a mile on the lowland of the same farm. It was found impossible to prevent the spread of virus diseases and the consequent degeneration of the stocks, under sheltered lowland conditions, by "rogueing out" diseased plants and their healthy neighbours (3), but that this could be accomplished by lifting tubers for seed purposes before the end of July (3).

There was thus good reason for the belief that, with suitable precautions, stocks of potatoes could be kept free, or almost free, from virus

diseases in many localities in North Wales. It was realised, throughout this preliminary work, that factors other than freedom from virus diseases might be essential for good seed production. The short growing season in the north of Scotland and the consequent immaturity of the seed stocks, for instance, has long been suggested as a partial explanation of the superiority of Scotch seed. This has been finally disposed of by chequer-board trials in which fully mature healthy seed gave as good a yield as immature healthy seed (4). By similar trials it has also been proved that virus diseases alone are responsible for degeneration, or that if other factors are involved, they fluctuate with virus diseases and for practical purposes can therefore be ignored (5).

#### COMMERCIAL SEED POTATO SCHEMES.

An abortive scheme for testing commercial possibilities in 1923 was resuscitated in 1927 and formed the basis of a larger scheme started in

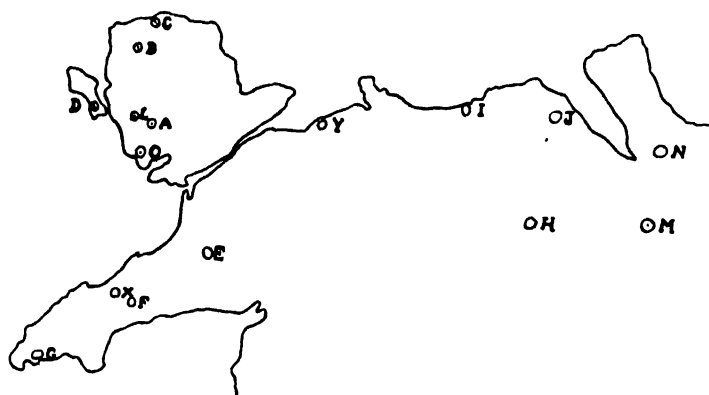


Fig. 1. Distribution of seed-potato producing centres referred to in text.

1928, which was made possible by a grant from the Ministry of Agriculture and Fisheries. The object was to test the value of seed produced in certain localities (see Fig. 1), under the close supervision of the College. If successful, it was hoped that it would result in the development of a new industry in North Wales for the commercial production of seed. In the selection of the centres regard was paid to the suitability of the grower as well as of the locality, for it was conditional to admittance to the scheme for the grower to undertake (a) to grow only the healthy stocks provided and to maintain these without change, for at least three years, (b) to adopt any method required for the maintenance of the health and vigour of the stocks, and (c) to supply a small quantity of seed each year

for experimental purposes. The seed supplied to the growers under the 1927 scheme was from a reliable source in Banffshire, whilst those joining the scheme in 1928 were provided with certified Athlone seed, one variety (Kerr's Pink) of which had been propagated from tested units under the direction of Prof. P. A. Murphy and the Irish Free State Department of Agriculture.

When the schemes were drawn up by one of the writers it was anticipated that few, if any, of the centres would be as favourably placed for seed production as, for instance, certain localities in the north of Scotland in which little or no transmission of virus diseases occur even when diseased plants are allowed to mature in the drills; any such centres were to be placed in Class I. Class II would include centres where seed stocks might be maintained over a long period by adopting precautions, *e.g.* "rogueing out" diseased plants, to prevent the spread of virus diseases. Similar conditions apply to the bulk of the seed producing areas in Scotland. Class III centres would be those only able to maintain certain of the more resistant varieties for seed purposes, or possibly less resistant ones for a short period of years when exceptional precautions (*e.g.* early lifting of seed stocks for subsequent use on their own farms) were taken to prevent virus infection.

#### FIELD OBSERVATIONS.

During each of the past four years at least three, and in most cases four, visits of inspection were made to each centre. Every plant was examined separately for health and identity, and any rogues or unhealthy plants were immediately removed. Great importance was attached to the date of these inspections and two were usually made before the end of June to ensure the removal of all sources of infection before aphid infestation became general; further inspections being made in July and August.

Table I shows the incidence of virus infection at the centres in 1928. Very little disease was found in the first ten centres in which Irish seed was planted for the first time; the most heavily infected crop having only 0.48 per cent. disease present. The five centres in which Scotch seed was used were in their second year, and it will be noted that three of them showed only 0.3 per cent. virus infection. At one centre 2.58 per cent. disease developed, due almost certainly to a diseased stock of Majestic having been introduced contrary to the agreement made at the beginning of the scheme.

In another centre the plants were infested with eelworm, and as the symptoms closely resemble those of leaf-roll, the figures given in Table I



Table I.  
*Incidence of virus diseases in 1928.*

Centre	Year admitted into scheme	Sharpe's Express				Great Scot				Kerr's Pink				All varieties			
		Leaf-roll %	Mosaic %	Total %		Leaf-roll %	Mosaic %	Total %		Leaf-roll %	Mosaic %	Total %		Leaf-roll %	Mosaic %	Total %	
A	1928	0.19	0.25	0.44		0.08	0.21	0.29		0.09	0.09	0.18		0.12	0.18	0.30	
B	"	0.53	0.04	0.57		0.18	0.09	0.25		0.11	0.12	0.23		0.26	0.06	0.34	
C	"	0.35	—	0.35		0.10	0.12	0.22		—	0.11	0.11		0.15	0.07	0.22	
D <sub>1</sub>	"	—	0.30	0.30		—	—	—		No Kerr's Pink				—	0.15	0.15	
E	"	0.35	0.15	0.50		0.12	0.11	0.23		0.15	0.07	0.22		0.20	0.11	0.31	
F	"	0.13	—	0.13		0.13	0.32	0.45		0.10	0.17	0.27		0.12	0.16	0.28	
G	"	0.06	0.38	0.46		0.11	0.33	0.44		0.07	0.25	0.32		0.08	0.32	0.40	
H	"	0.14	0.36	0.50		0.18	0.15	0.33		0.13	0.19	0.32		0.15	0.23	0.36	
I	"	0.11	0.33	0.44		0.19	0.10	0.29		0.13	0.08	0.21		0.14	0.17	0.31	
J	"	0.38	0.38	0.76		0.24	0.13	0.37		0.22	0.11	0.33		0.28	0.20	0.46	
J <sub>2</sub>	1927	No Sharpe's Express				0.14	0.06	0.22		0.23	0.17	0.39		0.18	0.12	0.30	
L	"	"	"	"		0.20	0.05	0.25		0.22	0.14	0.36		0.21	0.09	0.30	
M	"	"	"	"		0.10	0.07	0.17		0.34	0.10	0.44		0.22	0.08	0.30	
N	"	"	"	"		0.45	0.83	1.28		0.91	0.97	3.86		1.66	0.80	2.56	
O	"	"	"	"		0.93	0.16	1.09		2.03	0.61	2.64		1.46	0.38	1.86	

Note. A "dash" (—) = no disease.

(Centre O) probably represent the combined effects of the eelworm and leaf-roll. At all the centres the varieties were very pure, *i.e.* true to type, except the Irish Great Scot which contained almost 3 per cent. of rogues, some being of "wart susceptible" varieties.

Similar results were obtained in 1929 as is shown in Table II. There was, however, an extraordinary increase in leaf-roll infection in the variety Great Scot at one centre, where 4.18 per cent. of disease was recorded. Although every suspiciously rolled plant was at once removed, there was a significant rise in the following year to 60 per cent. and the centre has, of course, been discarded from the scheme.

The 1930 observations were regarded as critical since sufficient time had elapsed to disclose evidence of degeneration in the stocks, which were now three and four years old. Table III summarises the field observations in that year and it will be seen that at eight centres less than 0.5 per cent. total virus disease was present. The stocks at these eight centres were therefore as healthy as when first introduced into Wales, and there seems to be no reason why healthy seed should not be produced under such conditions. At three centres there was a slight increase in infection, but in no case did this exceed 3 per cent. One of the writers has never found a significant fall in yield in a crop containing less than 6 per cent. of leaf-roll, so that seed of considerable value is still being produced at these three centres. At the remaining two centres the rise in infection (13 per cent. and 33 per cent.) definitely eliminates them from the scheme.

#### *Yield trials.*

The vigour of the stocks at all the more successful centres would be difficult to excel, but yield trials, of course, were indispensable as a test of the value of these stocks for seed purposes. The seed of each variety was obtained from the centres in the early winter of 1929, this being mixed in equal proportions from ten centres to constitute the Welsh seed used for the trials. The Scotch seed for the trials was obtained from the north of Scotland at the same time as the Welsh seed, and both lots were boxed under identical conditions until planting time. For the College Farm trial Scotch "stock" certified seed was used, this carrying with it a guarantee of complete absence of "rogue" varieties and of all diseases of the foliage—including virus diseases. Since "stock" seed of the variety Sharpe's Express was not available, a specially selected strain was obtained and used in this trial. Trials were also laid down in a number of counties throughout Wales; the Welsh seed in these cases being tested against ordinary T.S. Certified seed from Banffshire.

Table II.  
*Incidence of virus diseases in 1929.*

Centre	Year admitted into scheme	Sharpe's Express			Great Scot			Kerr's Pink			All varieties		
		Leaf-roll %	Mosaic %	Total %	Leaf-roll %	Mosaic %	Total %	Leaf-roll %	Mosaic %	Total %	Leaf-roll %	Mosaic %	Total %
A	1928	0.21	0.12	0.33	0.21	0.05	0.26	0.14	0.06	0.20	0.18	0.08	0.26
B	"	0.15	0.03	0.18	0.81	0.02	0.83	0.07	0.08	0.15	0.34	0.04	0.38
C	"	0.12	0.10	0.22	0.21	—	0.21	0.03	0.03	0.06	0.12	0.04	0.16
D <sub>1</sub>	"	0.32	0.07	0.39	0.37	0.09	0.46	—	—	—	0.34	0.06	0.42
D <sub>2</sub>	"	0.14	0.14	0.28	0.10	0.04	0.14	—	—	—	0.06	0.06	0.14
E	"	0.16	0.24	0.40	0.17	0.04	0.21	0.04	0.13	0.17	0.12	0.14	0.26
F	"	0.15	0.14	0.29	0.20	0.14	0.34	—	—	—	0.12	0.11	0.23
G	"	0.08	0.07	0.15	0.57	—	0.57	0.06	0.05	0.06	0.23	0.10	0.33
H	"	0.34	0.06	0.40	4.14	0.04	4.18	0.24	0.09	0.33	1.57	0.06	1.63
I	"	0.06	0.06	0.12	0.62	0.03	0.65	0.10	0.13	0.23	0.30	0.06	0.36
J	"	0.20	0.08	0.28	0.14	0.06	0.22	0.02	0.04	0.06	0.08	0.06	0.14
L	1927	No Sharpe's Express	"	"	0.05	0.03	0.08	1.09	—	1.09	0.57	0.01	0.58
M	"	"	"	"	0.15	—	0.15	0.34	0.03	0.37	0.24	0.02	0.26
N	"	"	"	"	0.96	0.14	1.10	2.18	0.17	2.35	1.57	0.15	1.72
O	"	"	"	"	Discarded owing to eelworm infested land								

Note. A "dash" (—) = no disease.

Table III.  
*Incidence of virus diseases in 1930.*

Centre	Year admitted into scheme	Sharpe's Express			Great Scot			Kerr's Pink			All varieties		
		Leaf-roll %	Mosaic %	Total %	Leaf-roll %	Mosaic %	Total %	Leaf-roll %	Mosaic %	Total %	Leaf-roll %	Mosaic %	Total %
A	1928	0.12	0.07	0.19	0.33	0.06	0.39	0.04	0.05	0.09	0.16	0.06	0.22
B	"	0.14	0.18	0.32	0.16	—	0.16	0.07	0.03	0.10	0.12	0.07	0.19
C	"	0.15	0.19	0.34	0.25	0.12	0.37	0.30	0.02	0.32	0.23	0.11	0.34
D <sub>1</sub>	"	0.21	0.12	0.33	0.09	0.03	0.12	—	—	—	0.15	0.07	0.22
D <sub>2</sub>	"	0.16	0.10	0.26	0.12	—	0.12	0.10	0.10	0.20	0.10	0.06	0.29
E	"	0.09	0.07	0.16	0.03	0.12	0.15	0.13	0.03	0.16	0.10	0.05	0.15
F	"	0.05	0.06	0.13	0.34	0.06	0.43	—	—	—	0.14	0.06	0.23
G	"	0.88	0.06	0.96	1.87	—	1.87	1.90	—	1.90	1.55	0.02	1.57
H	"	9.00	*	9.00	60.00	*	60.00	30.00	*	30.00	33.00	*	33.00
I	"	7.90	*	8.16	20.10	*	20.10	10.80	*	10.80	12.90	*	12.90
J	1927	No Sharpe's Express	"	"	0.03	0.24	0.27	0.06	0.01	0.07	0.04	0.13	0.17
L	"	"	"	"	0.59	0.09	0.68	4.14	0.14	4.28	2.36	0.12	2.48
M	"	"	"	"	0.88	0.14	1.02	1.65	0.16	1.81	1.36	0.15	1.41
N	"	"	"	"	2.72	—	2.72	4.78	—	4.78	3.70	—	3.70
O	"	"	"	"	Eelworm infestation confirmed, and centre finally discarded								

Note. A "dash" (—) = no disease.

\* The high percent. leaf-roll infection at centres I and J definitely eliminated them from the scheme, so that percent mosaic infection was not determined.

Table IV.  
*Welsh seed potato trials, 1930.*

	Yield in tons per acre		Difference in yield favouring		Proportion of ware seed and chate to total crop	
	Welsh seed	Scotch seed	Welsh seed	Scotch seed	Welsh seed	Scotch seed
<i>Sharpe's Express:</i>						
Aber (average of 8 plots)	15.60	15.02	0.58	—	77.16. 7	78.16. 6
Anglesey: Trefri*	7.65	5.23	2.42	—	38.54. 8	58.39. 3
Porthamel	8.85	7.98	0.87	—	20.63.17	35.54.11
Caernarvon: Madryn*	10.86	6.40	4.46	—	81.16. 3	84.13. 3
Denbigh: Llysfasi	7.45	5.96	1.49	—	42.42.16	52.32.16
Borras	13.58	13.98	—	0.40	47.43.10	56.35. 9
Flint: Padeswood	12.55	12.40	0.15	—	47.39.14	66.25. 9
Little Aston	4.35	4.55	—	0.20	90.10	96. 4
Carmarthen: Pibwrlwyd	8.14	8.31	—	0.17	43.49. 8	57.40. 3
Average	10.07	9.74	0.33	—	50.40.10	60.33. 7
<i>Great Scot:</i>						
Aber (average of 8 plots)	15.92	15.10	0.82	—	85.10. 5	88. 8. 4
Anglesey: Trefri	8.35	8.85	—	0.50	51.42. 7	63.28. 9
Porthamel	11.97	13.45	—	1.48	70.23. 7	73.20. 7
Caernarvon: Madryn	14.94	15.38	—	0.44	89. 8. 3	90. 7. 3
Plas Gwyn	12.75	12.81	—	0.06	75.18. 7	77.17. 6
Denbigh: Llysfasi	12.46	12.46	—	—	73.20. 7	79.16. 5
Borras	16.59	14.85	1.74	—	80.16. 4	83.14. 3
Flint: Padeswood	18.20	16.65	1.55	—	65.25.10	73.18. 9
Little Aston	13.0	13.05	—	0.05	92. 8	77.23
Monmouth: Usk	17.50	18.26	—	0.76	81.14. 5	72.23. 5
Glamorgan: Tregroes	14.45	14.59	—	0.14	89.11	92. 8
Carmarthen: Pibwrlwyd*	14.04	10.03	4.01	—	42.53. 5	56.42. 2
Average	14.19	14.13	0.06	—	71.23. 6	75.20. 5
<i>Kerr's Pink:</i>						
Aber (average of 8 plots)	15.10	15.65	—	0.55	84.11. 5	84.12. 4
Anglesey: Trefri	8.72	8.39	0.33	—	57.36. 7	59.36. 5
Porthamel	13.97	14.88	—	0.91	77.19. 4	77.17. 6
Caernarvon: Madryn*	16.46	13.26	3.2	—	85.11. 4	89. 9. 2
Plas Gwyn	13.09	13.04	0.05	—	78.21. 1	81.14. 5
Denbigh: Llysfasi	11.38	11.21	0.17	—	68.28. 4	73.24. 3
Borras	15.34	14.98	0.36	—	74.20. 6	80.16. 4
Flint: Padeswood	14.55	13.45	1.10	—	76.18. 6	83.13. 4
Little Aston	10.05	10.80	—	0.75	96. 4	90.10
Monmouth: Usk	14.51	13.68	0.83	—	76.20. 4	71.24. 5
Glamorgan: Tregroes*	13.04	16.48	—	3.44	76.24	89.11
Carmarthen: Pibwrlwyd*	15.49	10.02	5.47	—	48.48. 4	41.47.12
Average	12.96	12.89	0.07	—	72.23. 5	74.21. 5

\* Since at the majority of centres there was little difference between the Scotch and Welsh seed, it must be assumed that these results are abnormal, and they are not included in the general average.

At the College Farm separate plots were given to each variety and Welsh and Scotch "stock" seed were replicated eight times in alternate drills of 54 tubers each. The two end plants in each drill were discarded at lifting time, so that each unit drill contained 50 plants. In the variety Sharpe's Express misses occurred frequently, but equally so in the Welsh

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and Scotch seed, and the unit drill had to be reduced to 34 plants. The plots were kept under observation during the growing season, but at no time could any difference in vigour or foliage features be detected. The following are the results obtained:

### *Sharpe's Express.*

Welsh seed	—Mean total yield per 34 plants	= 76.13 ± 1.14 lb.
Scotch seed	— " "	= 73.12 ± 1.50 lb.
Difference in favour of Welsh seed = 3.01 lb., which is less than twice the p.e. to which it is exposed, i.e. ± 1.8 lb.		

### *Great Scot.*

Welsh seed	—Mean total yield per 50 plants	= 115.87 ± 1.46 lb.
Scotch seed	— " "	= 109.50 ± 1.87 lb.
Difference in favour of Welsh seed = 6.37 ± 2.3 lb.		

### *Kerr's Pink.*

Welsh seed	—Mean total yield per 50 plants	= 109.50 ± 1.15 lb.
Scotch seed	— " "	= 113.25 ± 1.50 lb.
Difference in favour of Scotch seed = 3.75 ± 1.8 lb.		

It seems clear from the yields obtained that the Welsh seed was in no way inferior to that of the best obtainable Scotch seed.

The County Trials were carried out under the supervision of the County Organisers. All the trial plots were visited, and here again it was impossible to distinguish the two sources of seed from an examination of the growing crop. Table IV summarises the results obtained in these County Trials. There was little to choose from in the two classes of seed at most of the trial centres, what difference was found being in favour of the Welsh seed. In some cases a difference of over two tons occurred, but these are regarded as abnormal and are not included in the general average. Briefly, the trials throughout Wales confirm those secured at the College Farm and it must be concluded that the Welsh seed is of equal merit to a good stock of Scotch seed.

### THE APHID VECTOR PROBLEM.

The relation of aphides to the spread of virus diseases has become commonplace in literature and it is evident that no study of the conditions necessary for seed potato production would be complete without a thorough examination of the vector problem. Despite the amount of work that has been carried out under glasshouse and caged conditions there is a surprising lack of information concerning the biology and ecology of potato aphides under natural field conditions—a significant gap when the factors influencing seed potato production are under investigation. In an endeavour to bridge this gap and in order to ascertain the most

Table V.  
*Incidence of aphid infestation in 1928.*

Centre	Date	Sharpe's Express			Great Scot			Kerr's Pink			Species of aphides in sample
		I	II	III	I	II	III	I	II	III	
B	Aug. 18th	—	—	—	100-0	25-0	0-44	—	—	—	<i>M. gei</i>
C	"	—	—	—	100-0	64-0	1-76	—	—	—	<i>M. gei</i> ; <i>Caranella cauae</i>
D	Aug. 2nd	—	—	—	100-0	—	22-1	100-0	—	20-1	<i>M. gei</i> ; <i>M. pisi</i>
D	Aug. 20th	—	—	—	80-0	—	0-61	—	—	—	<i>M. gei</i> ; <i>M. persicae</i> ; <i>M. concoloratus</i>
E	July 12th	18-0	—	Neg.	24-0	—	Neg.	12-0	—	Neg.	<i>M. gei</i> ; <i>M. persicae</i>
F	July 11th	75-0	—	"	68-0	—	"	76-0	—	"	<i>M. gei</i> ; <i>M. persicae</i>
G	"	18-0	8-0	0-12	16-0	Neg.	"	8-0	Neg.	"	<i>M. gei</i> ; <i>M. persicae</i>
G	Aug. 24th	—	—	—	100-0	72-0	4-5	100-0	79-0	3-3	<i>M. gei</i> ; <i>M. persicae</i>
H	July 17th	58-0	—	1-5	*16-0	—	0-2	48-0	—	0-5	<i>M. gei</i> ; <i>M. pseudosolanis</i> ; <i>Drepanosiphum aceris</i>
I	Aug. 8th	—	—	—	84-0	47-0	1-46	86-0	50-0	1-43	<i>M. gei</i> ; <i>M. persicae</i>
J	"	—	—	—	100-0	63-0	3-6	100-0	64-0	2-52	<i>M. gei</i> ; <i>M. persicae</i>
X	July 12th	34-0	—	Neg.	62-0	—	Neg.	42-0	—	Neg.	<i>M. gei</i> ; <i>M. persicae</i>
Y	Aug. 15th	—	—	—	100-0	82-0	6-42	—	—	—	<i>M. gei</i> ; <i>M. persicae</i>

I = Percentage of plants infested with aphides.

II = Percentage of leaves infested with aphides.

III = Average number of aphides per leaf. (Total leaves, not infested leaves.)

Neg. = Negligible.

\* Great Scot were situated at the summit of the field which had a very marked slope.

promising lines of development a general survey of the potato aphides was made during 1928 at the eleven prospective seed potato centres. The method ultimately adopted to obtain comparable results within a minimum period was as follows: at each centre the aphid infestation on each variety was ascertained separately. The individual leaf was the unit taken for observation and a random count was made on leaves on 100 different plants. During the count the crop was traversed once up and down, thus dividing the counts into two lots of 50 leaves. If the difference in the number of infested leaves in the two sets was greater than 5 a recount was made and the average taken. Care was taken that the leaves examined occupied varying positions on the plants. The counts from the different varieties were compared and any apparently well-marked varietal difference in infestation was checked by confirmatory counts. Table V summarises the results obtained.

During the years 1921-7 Walton<sup>(7)</sup> showed that at certain centres in North Wales the potatoes, when examined, were free from aphid attack; at other centres the infestation was comparatively slight, and observations stressed the importance of shelter as a factor governing aphid infestation. It thus appeared probable that such centres would produce healthy seed potatoes and hence in the present scheme bleak, exposed centres were chosen as prospective seed-producing farms.

#### OBSERVATIONS ON THE APHID INFESTATION IN 1928.

The results of the 1928 survey were thus somewhat unexpected. Although the centres had been chosen for their bleak, exposed situations, it is seen from Table V that at six centres 100 per cent. of the plants were infested, while only one centre had less than 50 per cent. of the plants attacked by aphides. As many as 79 per cent. of the leaves were infested at one centre, while the average number of aphides per leaf was also high, as for example at Centre D. This is considered the most exposed of the centres, being on the west coast of Anglesey about 100 yards from the sea, yet an average of 22 aphides per leaf was taken. It is apparent, therefore, that a moderately heavy infestation of aphides occurred at all the selected seed potato centres in 1928.

General field observations in the province suggested that in the case of the Flintshire, Denbighshire and Anglesey centres the period of maximum infestation occurred from the middle to the end of July. Centre D, which was visited on two occasions, showed, on August 2nd, 100 per cent. of the plants infested and an average of 22 aphides per leaf, but when revisited on August 20th the plant infestation had fallen to 80 per cent.

while there was an average of only 0.6 aphides per leaf. In South Caernarvon the period of maximum infestation was later; thus at Centre G, on July 11th, 18 per cent. of the plants were infested and less than 10 per cent. of the leaves, but on August 24th, 100 per cent. of the plants were infested and 72 per cent. of the leaves with an average of 4.5 aphides per leaf.

Representative samples of the aphides were collected at each centre and the specimens later identified. Acknowledgments are due to the late Prof. Theobald for kindly confirming these identifications. *Macrosiphum gei* Koch predominated at all centres, whilst *Myzus persicae* Sulz was second in order of prevalence and was included in the samples taken at eight out of the eleven centres. *Myzus pseudosolani* Theo. was also included at two of the centres. Other species which apparently were stray infestations were *Cavariella capreae* Fab., *Drepanosiphum acerina* Wlk. and *Myzus convolvuli* Koch<sup>1</sup>.

When the infestation of aphides was slight it was seen that the variety Kerr's Pink was least attacked, but no such varietal resistance was observed when the infestation of aphides was high.

#### OBSERVATIONS IN 1929.

In 1929 attention was concentrated on six of the centres, from which aphides were collected for experimental purposes to which reference will be made later. In view of the observations of 1928 an earlier date was chosen for the visits. Table VI summarises the results obtained in 1929, and it is seen that during mid-July 100 per cent. of the plants at four centres (D, I, J, X) were infested with aphides, while at two centres (I, J) 100 per cent. of the leaves also were infested, and the average number of aphides per leaf was 30 and 35 respectively. These observations thus confirmed those of the previous year in that these centres, specially chosen for their bleak, exposed situations, were liable to heavy infestations with aphides. The relative prevalence of *Macrosiphum gei* and *Myzus persicae* was similar to that of 1928, and, in addition, the indication of a varietal resistance of Kerr's Pink to aphid attack—only when the general infestation is slight, was confirmed.

#### OBSERVATIONS IN 1930.

In order to ascertain more closely the period of maximum infestation, it was decided to make two visits to each centre, one in mid-July and the other in mid-August. It will be seen from Table VII that a heavy infestation of aphides took place in 1930, for at five centres (D, G, H, I, J)

<sup>1</sup> Mr Laing, of the British Museum, now considers this species to be a synonym of *Myzus pseudosolani*.



Table VI.

*Incidence of aphid infestation in 1929.*

Centre	Date	Sharpe's Express			Great Scot			Kerr's Pink			Species of aphid <i>M. gei</i> ; <i>M. persicae</i>
		I	II	III	I	II	III	I	II	III	
D	July 17th	100-0	44-0	1-6	100-0	55-0	2-41	—	—	—	<i>M. gei</i> ; <i>M. persicae</i>
G	July 13th	20-0	11-0	0-18	24-0	14-0	0-24	15-0	5-0	0-11	
H	July 15th	80-0	51-0	1-63	76-0	50-0	1-43	60-0	41-0	1-14	
I	July 16th	—	—	—	100-0	100-0	35-4	100-0	100-0	28-6	
J	"	100-0	100-0	30-0	100-0	100-0	19-1	100-0	100-0	18-7	
X	July 18th	100-0	65-0	2-4	100-0	59-0	1-36	100-0	62-0	2-48	"

I = Percentage of plants infested with aphides.

II = Percentage of leaves infested with aphides.

III = Average number of aphides per leaf. (Total leaves, not infested leaves.)

Table VII.

*Incidence of aphid infestation during July and August 1930.*

Centre	Date	Sharpe's Express			Great Scot			Kerr's Pink			Prevalence of <i>M. gei</i> and <i>M. persicae</i>
		I	II	III	I	II	III	I	II	III	
D	July 30th	100-0	64-0	1-2	100-0	70-0	2-26	100-0	62-0	1-24	75 % <i>gei</i> 80 % "
G	July 17th	100-0	25-0	0-3	100-0	40-0	0-64	100-0	28-0	6-2	
H	July 15th	100-0	86-0	3-17	100-0	60-0	1-42	100-0	64-0	1-5	
I	"	—	—	—	100-0	33-0	3-24	100-0	62-0	61-0	
J	"	100-0	100-0	35-2	100-0	100-0	36-8	100-0	100-0	30-0	
X	July 17th	—	—	—	60-0	34-0	0-44	60-0	18-0	0-34	75 % " 80 % " much higher
D	Aug. 19th	—	—	—	—	—	—	75-0	20-0	0-5	
G	Aug. 16th	—	—	—	100-0	26-0	0-4	100-0	25-0	0-3	
H	Aug. 12th	—	—	—	75-0	22-0	0-3	75-0	18-0	0-2	
I	"	—	—	—	50-0	35-0	0-5	50-0	42-0	0-6	
J	"	—	—	—	70-0	38-0	0-6	75-0	32-0	0-4	"
X	Aug. 16th	—	—	—	100-0	84-0	2-5	100-0	72-0	1-8	

I = Percentage of plants infested with aphides.

II = Percentage of leaves infested with aphides.

III = Average number of aphides per leaf. (Total leaves, not infested leaves.)

in mid-July 100 per cent. of the plants were attacked and the leaf infestation varied from 25 to 100 per cent. at all six centres. The second visit made during mid-August showed that at four centres (D, H, I, J) in Flintshire, Denbighshire and Anglesey, the infestation was markedly reduced, but again at the two centres (G, X) in South Caernarvonshire the infestation had been maintained, and even increased.

The possibility of a specific relationship between species of aphides and transmission of virus diseases, which has been suggested of late, prompted more detailed observations to determine the relative incidence of the more prevalent species. During 1930 an opportunity was not forthcoming for really critical determinations, but in the time available sufficient data were obtained to leave no doubt of the predominance of *Macrosiphum gei* at all centres in mid-July. *Myzus persicae*, however, occurred at all centres and comprised from 10–25 per cent. of the aphid population. By mid-August the numbers of *Macrosiphum gei* had fallen very considerably and now represented about half the numbers present. This of course increased the proportion of *Myzus persicae* although in numbers they were not markedly different from those found a month earlier. The maximum infestation with *Macrosiphum gei* was rapidly attained, at the eastern centres, about the middle of July and this was followed by an equally rapid decline in numbers. *Myzus persicae*, on the other hand, slowly reached maximum intensity which, in comparison with *Macrosiphum gei*, was very low; this was followed by an equally slow decline. *Myzus pseudosolanii*, when present, was found only in relatively small numbers. At all centres the proportion of alate forms of all species was very small.

It was observed with reference to varietal reaction that although Kerr's Pink showed a lighter infestation than the other varieties at three centres this apparent varietal resistance was not consistently maintained, and it was evident that in practice other factors, such as intensity of infestation, situation in field and age of crop, masked any varietal reaction shown by any of the three varieties grown.

#### OBSERVATIONS IN 1931.

Observations were made on the variety Great Scot at all six centres when all aphides observed were individually identified; the results are given in Table VIII. Four of the centres were visited more frequently and over a longer period of the growing season, and it was thus possible to confirm the conclusions of previous years concerning the period of maximum infestation.

Table VIII.

*Infestation of aphides at centres in 1931.*

Variety: Great Scot.

Centre	Date	Plant	Leaf	Percentage infestation	Average no. of aphides per leaf	Species per 100 leaves		
						<i>M. gei</i>	<i>M. persicae</i>	<i>M. pseudo-solani</i>
D	June 24	10	0.3	0.53	5		0.3	Nil
D	July 9	62	20	0.71	64 (1)		7	"
D	July 17	75	42	1.94	134		50	"
D	Aug. 17	60	10	0.27	3		24 (2)	"
G	June 17	Nil	Nil	Nil	Nil		Nil	Nil
G	July 16	1	0	0.09	9		"	"
G	Aug. 13	50	28	0.63	10		53	"
H	July 20	72	22	0.12	8 (1)		4	"
H	Aug. 20	44	14	0.22	1		20	1
I	June 9	Nil	Nil	Nil	Nil		Nil	Nil
I	July 15	100	46	0.94	73 (2)		21	"
I	Aug. 12	100	64	0.8	20 (1)		45 (1)	12
J	June 9	Nil	Nil	Nil	Nil		Nil	Nil
J	July 6	50	13	0.35	22		23	"
J	July 15	100	53	1.58	89 (1)		69	"
J	Aug. 12	82	32	0.74	22		46 (1)	6
X	June 17	Nil	Nil	Nil	Nil		Nil	Nil
X	July 16	2	0	0.03	1		2	"
X	Aug. 13	53	16	0.26	5		21	"

Figures in brackets in columns 6 and 7 = number of alate forms.

At Centre J, which has proved unsatisfactory for the production of seed potatoes owing to the marked increase of virus diseases, no aphides were found on June 9th whereas 50 per cent. of the plants were infested on July 6th. On July 15th 100 per cent. of the plants were infested and 53 per cent. of the leaves attacked with an average of 1.58 aphides per leaf. On August 12th the leaf infestation had fallen to 32 per cent., with an average of only 0.74 aphides per leaf.

At Centre D, one of the most successful centres in the Scheme, the leaf infestation was only 0.5 per cent. on June 24th, 20 per cent. on July 9th, increased to 42 per cent. by July 17th and subsequently declined to 10 per cent. by August 17th. At this centre the leaves died off before the end of July and on August 17th only 20 per cent. of the leaves of Kerr's Pink remained green.

At Centre H, where only two visits were made, a similar state of affairs occurred, while at the College Farm, Aber, where weekly observations were being made, the maximum numbers of aphides were present during the week July 16th to 23rd. Centre I did not show a marked reduction in infestation by August, but this centre would not have been included except for continuity of records for, this year, the area of

potatoes only comprised a few rows near the hedge alongside a main road—certainly an abnormal field environment for the aphid populations.

At the two South Caernarvonshire centres (G, X) the infestation was again markedly contrasted with the eastern centres, a practically negligible infestation being recorded in mid-July followed by a definite increase up to mid-August. At these centres again the leaves died off early.

The observations of 1931, therefore, confirmed the period of maximum infestation of aphides and also emphasised the delay in infestation which occurred at centres G and X.

This season the species were segregated and the numbers of the different species present per 100 leaves, taken at random, are given in column 5 of Table VIII. It is clear, from the numbers of the respective species, that at all centres *Macrosiphum gei* rapidly attains maximum numbers and during mid-July it is the predominant species. Subsequently the numbers fall rapidly so that ultimately *Myzus persicae* is present in greater numbers than the former species. The curve of infestation by *Myzus persicae* is not quite so acute as that of *Macrosiphum gei* and the numbers attained by the latter species are never reached. *Myzus pseudosolani* was taken at three centres but not until August and then only in small numbers.

Again in 1931 the proportion of alate forms was exceedingly small.

#### INFECTIVITY OF THE APHIDES AT THE SEED-PRODUCING CENTRES.

The surprisingly heavy infestation of aphides at centres which were producing seed as free from virus diseases as when first introduced into Wales naturally opened up the question as to the extent to which aphides were acting as vectors. With this in view work was begun in 1929 which was shared equally by all three writers. A representative sample of aphides was collected at each of the six centres in mid-July 1929. In the laboratory they were transferred to sprouting half-tubers, suitably isolated in muslin cages, and, of course, the aphides from each centre were kept separate. After 14 days' infestation the tubers were fumigated and planted. Unfortunately the infestation was too severe and in the majority of cases the tubers failed to produce a plant. Those of Centre X alone grew successfully and gave evidence of leaf-roll transmission. The experiments were repeated in 1930 where the procedure was as follows: at the first visit to each centre in mid-July a representative sample of the aphid population throughout the crop was taken until approximately 100 individuals of mixed species had been obtained. They were conveyed to young growing plants produced from half-tubers, the sample from each

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centre being divided into three and placed on separate plants. The infested plants were isolated in aphid-proof cages as were also the half-tuber controls. After being allowed to feed on the plants for 14 days, the aphides were fumigated and the plants were transferred to aphid-proof glasshouses. A similar experiment was carried out with aphides from the same five centres in mid-August; the results of both experiments being tabulated in Table IX.

Table IX.

*Infectivity of aphides from five centres in July and August 1929.*

Centre	Date collected	Result	Date collected	Result
H	July 15	Transmission	Aug. 12	No transmission
J	"	"	"	Transmission
I	"	"	"	"
X	July 17	"	Aug. 16	"
G	"	"	"	"

*Note.* All control plants remained healthy.

From Table IX it is evident that the aphid population, both in mid-July and in August at all centres included individuals which were active vectors of leaf-roll.

Table X.

Centre	Date	Condition of plant yielding aphides	Number and species of aphides			Result
			<i>M. gei</i>	<i>M. persicae</i>	<i>M. pseudo-solani</i>	
J	July 15	L.R. (3)	42	36	0	Transmission in two plants. Plant 3 with 20 <i>gei</i> only—No transmission
J	"	H. (2)	82	43	0	No transmission
I	"	L.R. (1)	20	1	0	"
I	"	H. (2)	46	23	0	"
G	July 16	L.R. (2)	0	13	0	Transmission in both plants, but one control showed L.R.
G	"	H. (2)	0	8	0	No transmission
X	"	L.R. (2)	3	3	0	Transmission in both plants
X	"	H. (2)	6	6	0	No transmission
D	July 17	H. (2)	16	44	56	"
J	Aug. 12	L.R. (2)	4	6	3	Transmission in both plants
J	"	H. (2)	33	19	15	No transmission
I	"	L.R. (2)	20	1	9	Transmission in plant with 1 <i>persicae</i>
I	"	H. (3)	39	31	22	Transmission in 1 plant
G	Aug. 13	H. (3)	8	62	29	No transmission
X	"	H. (3)	26	70	15	"
D	"	H. (3)	14	72	9	"

L.R. = Leaf-roll. H. = Healthy.

Numbers in brackets = number of experimental plants.

In 1931 these experiments were carried a stage further when the samples of aphides were separately collected from leaf-roll plants and

from apparently healthy plants at each centre during two visits, one in mid-July and the other in mid-August. The aphides were taken into the laboratory, identified and divided into duplicate or triplicate lots according to the numbers available and placed on plants grown from half tubers. The plants before and after infestation were kept under aphid-proof conditions, as also were the corresponding control half-tuber plants. The results of these experiments are summarised in Table X.

The results confirm those of 1930 in that a representative sample of the aphid population at all these seed potato centres included active vectors of leaf-roll. In the 1931 experiments, however, with one exception, transmission only occurred when the aphides had been collected from leaf-roll plants in the field. Indeed, if *Myzus persicae* is considered alone, 347 individuals of this species taken from at least 250 different plants, which were apparently healthy, did not include any active vectors. On the other hand, cases of transmission occurred when only a single specimen of *Myzus persicae* from a leaf-roll plant was transferred to experimental healthy ones. This result would not have been surprising if it could have been assumed that little or no movement was taking place, but that this was far from being the case was shown by a study on the ecology of potato aphides, the results of which are to be published elsewhere.

The definite segregation of the species in these experiments provided interesting results. It was significant to note that in the plants associated with aphides from Centre J one plant which had 20 specimens of *Macrosiphum gei* only (taken from leaf-roll plants) failed to show transmission, whereas the other two plants which had *persicae* among the infesting aphides proved ultimately to have had leaf-roll transmitted to them. Again in the July experiments concerning Centre I, no transmission occurred with aphides taken from leaf-roll plants when the aphides consisted of 20 *gei* and 1 *persicae* (the solitary specimen of *persicae* died).

On the contrary, the aphides taken from leaf-roll plants from Centre X in July, which included in the one case 1 *persicae* and 2 *gei* and in the other 2 *persicae* and 1 *gei*, successfully transmitted leaf-roll. Further, in the August experiments concerning Centre I, when only one specimen of *persicae* was included in the sample taken from leaf-roll plants, the plant on which this specimen was placed showed transmission, whereas the other plant which was infested with 19 *gei* and 12 *pseudosolani* also from leaf-roll plants gave negative results.

## DISCUSSION.

The scheme appears to have established the fact that, with suitable supervision and precautions against the spread of virus diseases, seed potatoes of high quality can be produced on a commercial scale in certain localities in North Wales. Of the fifteen farms originally taking part in the scheme eight are producing seed which, in point of health and purity, is probably as good as any in commerce; they are regarded as good Class II farms (and possibly even Class I) in the sense used on p. 531 of this communication. Three more centres have shown only a light increase in infection with virus diseases, but as this does not in any case exceed 3 per cent. it is improbable that a crop grown from such seed would yield appreciably less than a perfectly healthy one. These centres are thus still producing quite valuable seed, and the farms may reasonably be included in Class III, *i.e.* those able to maintain stocks in a high state of health only when exceptional precautions are taken to prevent the spread of virus diseases. The remaining four farms have been discarded from the scheme, one because of the occurrence of eelworm in the soil, a second owing to the apathy of the grower, and in two cases because of the rapid spread of virus infection in the crop.

The situation of the centres under the scheme is of some interest. Of the eight most successful farms, four are in Anglesey and four in the Western part of Caernarvonshire. All have three features in common which, separately or collectively, may account for their relative freedom from virus diseases.

(1) They are situated within three or four miles of a bleak and exposed sea coast. Although the aphid infestation was higher than was anticipated the climatic conditions would tend to reduce the aphid population below the average for the province, particularly prior to the end of July.

(2) The type of farming practised in these areas is chiefly pastoral and potato fields are both small and relatively far apart. This gives the centres a form of isolation from outside sources of infection.

(3) The prevailing wind is from the sea and seems to cause a premature ripening of the crops; even the haulms of the variety Kerr's Pink are practically down by the end of August. Blight usually occurs earlier in these Western districts than in the East and the destruction of foliage by this disease will also tend to keep down aphid attack. Both premature ripening and the early incidence of blight are believed to have much the same effect on the health of the tubers as "early lifting" of seed.

Two of the less successful centres are of an inland and upland character;

one is at an elevation of 900 feet, the other at 500 feet. In spite of this altitude, however, they are more sheltered than the eight Class II farms. The two centres which have proved definitely unsuitable are still more sheltered, lowland farms, where isolation from other potato crops is less complete. The outline map on p. 530 shows the location of all the farms under the scheme.

The scarcity of aphid vectors of virus diseases is usually advanced as a sufficient explanation of the superiority of one area over another in respect of seed potato production. It is evident, however, that the measure of success obtained by certain centres under the present scheme cannot be explained so simply. At all the more successful centres at least 50 per cent. of the plants were infested with aphides at some period of the year. For instance, at one of the best centres (D) 100 per cent. of plants were infested with an average of 22 aphides per leaf in 1928, while again in 1929 and 1930 100 per cent. of plants were infested, and in the latter year as many as 70 per cent. of the leaves also were attacked by aphides, yet as an accumulative effect, only a negligible amount of leaf-roll appeared in the crops in 1931.

The absence of increase in leaf-roll at the more successful centres cannot be due to the lack of potential vectors, for the species *Myzus persicae* accounted for at least 25 per cent. of the aphid population at all centres. *Myzus pseudosolani* also occurred at most centres and this species is stated to transmit leaf-roll at least occasionally (8, 9). Even *Macrosiphum gei*, which constituted 75 per cent., cannot wholly be ignored (6, 8). Neither can an explanation be found in the non-infective condition of such vectors as *Myzus persicae*, since representative samples of aphides taken from all five centres, where infectivity was investigated, included active vectors of leaf-roll.

The problem is an intricate one which will require further investigation. All that can now be attempted is to suggest certain possibilities which appear to offer at least a partial solution of the problem. The maintenance of healthy seed potatoes apparently depends partly on the relation between the date of maximum aphid infestation of the crop and the stage of maturity of the foliage. It has already been pointed out that this date occurred later in the Western centres than in the Eastern ones and that the former included those centres where the crops matured early in the season and/or were prematurely cut down by blight. It is in these Western centres where seed potato production has been most successful.

At these centres the degree of infestation with aphides was not quite as high as in the East, but nevertheless was considerable. For instance



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at Centres G and X 100 per cent. plant infestation and up to 72 per cent. leaf infestation was recorded. But at these two centres the maximum infestation did not take place until mid-August when the leaves had matured. In addition, at Centre D 100 per cent. plant infestation was observed in July but here the plants matured unusually early each year and were also prematurely cut down by blight. Another factor is undoubtedly the degree of mobility of the aphid population within the crop. So long as there is no movement of aphides from plant to plant there can be no spread of virus diseases, no matter what species of aphides or number of infected plants are present. Anything tending to disturb the aphides is therefore of importance in facilitating the spread of disease. This aspect of the problem is being made the subject of further study.

The present work emphasises the need for critical studies on the biology of aphides and of the ecology in particular of the aphid population of potatoes. It is urgently required, since there is no reason to suppose that localities, equally capable of producing good seed potatoes as those described above, cannot be found in other parts of England and Wales. Even in Scotland the seed potatoes are not of uniformly high quality, and a study of the aphid vector problem north of the Tweed is particularly desirable. It is suggested that the aim in seed potato producing areas should be to segregate farms into classes of the nature suggested on p. 531, and to differentiate between these in awarding seed certificates.

The writers are indebted to Prof. R. G. White for his valuable assistance, much helpful criticism, and sustained interest in the scheme. Grateful acknowledgments are also due to all the County officials who carried out yield trials.

### SUMMARY.

1. Under a scheme, begun in 1927, and supported by a special grant from the Ministry of Agriculture since 1928, an attempt is being made to test the value of certain localities in North Wales for the production of seed potatoes.

2. Evidence is produced to show that out of fifteen farms originally selected for this purpose, eight have maintained their stocks without increase of virus diseases, three have shown only a slight increase, whilst four have been discarded from the scheme. Replicated yield trials at the College Farm proved that seed mixed from these eleven farms was equal to Scotch "stock" seed, and the value of this Welsh seed was confirmed by trials in a number of Welsh counties in which it was tested against ordinary T.S. Scotch seed.

3. Concurrently with this, a study has been made of the aphid population on the potato crops at these centres, which has led to the following conclusions:

(a) The absence of increase of virus diseases, and of leaf-roll in particular, at the more successful centres is not due to the scarcity of aphides, nor to the absence of known vectors of the diseases, such as *Myzus persicae*. Neither can it be attributed to the non-infective condition of the aphid vectors present at these centres since representative samples, taken from potato crops, transmitted leaf-roll to healthy plants under glass. With one exception no transmission occurred with any of the species of aphides when taken from apparently healthy plants in a partially diseased crop. In samples taken from leaf-roll plants transmission only occurred when they included *Myzus persicae*.

(b) The accumulated data suggest that the maintenance of health in potato stocks is influenced not merely by the relative abundance of aphides, but rather by the relation between the date of maximum infestation and the stage of maturity of the foliage. The more successful centres, each year, showed either a delayed maximum infestation and/or the foliage was cut down earlier than at the less successful centres. The relative movement of aphides within the crops at the different centres is certainly of importance, but this is conditioned by many factors which are still under investigation.

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(Received February 4th, 1932.)

# ON THREE NEW VIRUS DISEASES OF *HYOSCYAMUS NIGER*

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(With Plates XXVIII—XXX.)

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## INTRODUCTION.

THIS paper describes a group of virus diseases infecting commercially grown *Hyoscyamus niger* (Henbane). The observations were made in the fields of Messrs William Ransom and Son, Ltd., Manufacturing Chemists of Hitchin, Bedfordshire, to whom I am indebted for facilities in the field and for material for experiments.

*Hyoscyamus* is grown for the leaves and the flowers which are used for the manufacture of pharmaceutical preparations. The plant is grown as a biennial crop sown, as a rule, in October, though in a bad autumn it may be held over until February. In the first year of growth two leaf crops are taken in July or early August, and late September. In the second year a

flower crop is harvested in June but two or three rows are generally kept for seed until August. After the flower crop is taken the roots are ploughed in and a rotation crop, wheat, potatoes, sanfoin or sugar beet, is grown. Messrs. Ransom grow the *Hyoscyamus* in three large plots in such an order that there is always one plot in leaf, one plot in flower and one plot under the rotation crop. Two of the plots are in one large field and the other in a field about a mile away. During the year in which the plots were under observation, July 1930 to September 1931, which involved two growing seasons of the *Hyoscyamus*, the rotation crop was sugar beet.

#### METHODS.

All the plants used in the experiments, unless stated to be taken direct from the field, were grown from known virus-free seed under as nearly as possible insect-proof conditions. Direct inoculations were made by the needle method (scratching through a drop of fluid from macerated leaves) or by rubbing with cotton wool. The insect cultures were kept in a specially designed insectary in cellophane cages(1). Stock insects were reared on cabbage or spinach which do not take or carry the diseases. The infected stock cages were enclosed, for greater precaution, in cupboards of phospho-bronze gauze, and the cages were only opened in the small culture chamber, which adjoins the insectary but is completely sealed off from it. The culture chamber was thoroughly sprayed with either pyrethrin or nicotine solutions after each experiment.

The insects were transferred on sterile camel-hair brushes, each insect being taken from the infected plant on to filter paper and transferred with a second brush, to prevent the virus being carried by touch. Infected insects were placed in suitable numbers on batches of young seedlings, which were kept as long as the insects were alive in lamp-glass cages in the insectary until after spraying, when they were removed to heated insect-proof glasshouse chambers.

#### PRELIMINARY OBSERVATIONS.

For convenience the first field containing the two plots will be referred to as Field A, plots 1 and 2, and the second as Field B. The first disease was obtained from Field A, plot 1, in September 1930 during an investigation of a flea-beetle attack. It was noticed in this plot that some of the plants were stunted and necrosed sometimes with deformation of the leaves and "rosetting" of the habit. The crop was at the end of its first year just before harvesting. The diseased condition was at first attributed to the effects of the beetle attack but, when a closer examina-

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tion revealed mottling of the leaves and yellowing or "clearing" of the veins, experiments were devised to determine whether a virus disease were present.

*Experiment I.* Winter buds from the crown of the tap roots were grown in Rothamsted plots free from the beetle; some of these plants showed stunting and mottling as in the field and some were normal. Plate XXVIII, fig. 1, shows three plants of which two are diseased and one is normal. Juice from the stunted and normal plants were needle-inoculated into young healthy seedlings and those inoculated from the stunted plants showed symptoms of disease.

*Experiment II.* Leaves from infected plants in the field were macerated and inoculated into healthy seedlings grown at Rothamsted; these also produced definite symptoms.

In the spring of 1921 an attempt was made to obtain more infected plants from Field A, plot 1, but the field had been ploughed ready for the rotation crop and plot 2 was only newly planted. Specimens were therefore taken from Field B, the crop of which was already in its second year. Juice from these plants and from plants from Field A, inoculated into *Hyoscyamus* produced different symptoms. The two fields continued to give these distinct characteristic symptoms whenever specimens were taken throughout the thirteen months during which they were under observation. Later experiments showed that there were two groups of diseases. The first group, from Field A, plots 1 and 2, was found to consist of two components, a "vein band" and a "yellow mosaic" type which were called Hy. II and Hy. III respectively with a possible third which has been temporarily called "Green *Hyoscyamus* mosaic" (4). The second group (from Field B) contained apparently only one virus of the "ring spot" type which is known as Hy. IV.

### VIRUS DISEASES FROM FIELD A.

#### *Direct inoculation.*

#### *Hy. I.*

Hy. I was the name arbitrarily given to the disease, as it came from the field before any information was obtained as to whether it was caused by one or more viruses. Juice from the presumed infected leaves was inoculated by needle into a batch of young *Hyoscyamus* seedlings as already mentioned. The resulting symptoms took the form of dark green spots on the older leaves. A second series of inoculations was made from field plants into large batches of *Hyoscyamus* and tomato seedlings.

All the *Hyoscyamus* seedlings showed the same symptoms, but the tomatoes were different in their reactions. Six out of eight inoculated tomato seedlings showed no symptoms, but two produced symptoms of extreme stunting with dark coloration, blistering and deformation of the leaves (Plate XXVIII, fig. 2). When inoculated back into tomato, *Hyoscyamus* and tobacco, juice from these stunted plants gave the same result in tomato. In *Hyoscyamus* and tobacco it caused a violent "yellow mosaic" with a tendency to broad dark green bands along the veins, necrosis of the older leaves, and some deaths among the younger or weaker plants (Plate XXVIII, figs. 1 and 2).

The first suggestion arising from these observations was that Hy. I was a complex of two viruses, one producing mild "green" symptoms and one violent "yellow" symptoms. In order to distinguish these they were given numbers; Hy. II for the mild and Hy. III for the violent disease. It was supposed, despite the discrepant fact that the Hy. III only showed in tomato though it is also capable of completely masking Hy. II in *Hyoscyamus*, that they were associated in the field and would continually occur together in inoculation. This, however, did not happen. The disease (Hy. III) obtained from the original tomatoes remained throughout a long series of inoculations involving about 600 plants of various kinds, constant to the distinct type of symptom and to other properties, physical and physiological, which will be described later, but on no other occasion was recovered from the field. Also it did not appear in any plants inoculated from the original Hy. I in *Hyoscyamus* or from the subsequent inoculation with Hy. II.

Later observations on Hy. II showed that it apparently never produces symptoms in tomato. In *Hyoscyamus* and tobacco it produces "vein band" symptoms in the young leaves (Plate XXIX, figs. 3 and 4) which persist throughout life in tobacco but not in *Hyoscyamus*. In this plant they disappear and become, in the older leaves, a dark green irregular mottle such as is described for Hy. I. Subsequent inoculation from infected field plants gave rise to "vein band" symptoms in the same way, and it is possible that they also appeared in the original Hy. I inoculation but were not observed as they disappear rapidly in *Hyoscyamus*.

### *Hy. III.*

**Host range.** The detailed description of Hy. III is given first as it is more characteristic in its symptoms and properties than Hy. II, and therefore more easy to deal with. Also the symptoms are more definite

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and the percentage of infection is higher than with Hy. II. The percentage of infection by scratching (needle method) or by rubbing is very high and cases of failure to "take" are very rare unless the juice has been kept for some time or passed through fine filters. In addition to tomato, *Hyoscyamus* and tobacco it causes disease in the following hosts:

*Nicotiana glutinosa*—a systemic infection of vein band symptoms similar to those caused by Hy. II in tobacco, but tending to become fainter in the older leaves. A striking characteristic of the disease in *N. glutinosa* is the "breaking" of the flower which is normally self-coloured pink, but in plants infected with Hy. III is white striped with pink. The symptoms take longer to show in *N. glutinosa* than in any other host—about fourteen or fifteen days as compared with five to seven days.

*Nicotiana glauca*—violent necrosis with blistering of leaves and stunting.

*Petunia* sp.—yellowing of regions round veins with necrosis of the older leaves.

*Datura stramonium*—symptoms similar to those in *Hyoscyamus*—yellow mosaic with tendency to dark green vein bands and blistering.

Hy. III causes no disease in the following Solanaceae: *Solanum nodiflorum*, *S. dulcamara*, *S. melongena* (Egg plant), *Atropa belladonna*, *S. tuberosum* (Potato)—the following varieties: Arran Chief, Arran Victory, President, Epicure, Great Scot; or in the Cruciferae which were tested as food plants for aphids: *Reseda odorata* (Mignonette), *Raphanus raphanistrum* (Radish), *Brassica oleracea* (Cabbage), *B. rapa* (Turnip). Two garden plants were also tested with negative results, viz. *Myosotis sylvaticum* and *Ageratum officinalis*.

*Symptoms.* In type the symptoms of Hy. III disease resemble those of the common tomato and tobacco mosaic diseases—aucuba (2), tobacco mosaic, and yellow mosaic of tobacco (Johnson, 6). The symptoms of Hy. III in tomato slightly resemble those of tobacco mosaic, but are more violent and the stunting is much more severe. Hy. III tomatoes present a characteristic "parsley head" appearance, in which the leaves are distorted into indefinite curly masses. (More detailed photographs of symptoms will be published in a later paper.) In tobacco the mosaic produced by Hy. III virus is more definitely yellow than tobacco mosaic but resembles the "yellows" disease. The type of symptoms shown in Plate XXIX, fig. 2, with the broad blistered dark green bands is characteristic of the younger plant. Later the bands become smaller and often necrotic at the edges, and the leaf becomes a confused chequered design of dark bands, yellow mottle and necrotic spots. In the old tobacco plant the

young leaves often "grow out" of the symptoms and appear to be normal though the virus can be recovered from them apparently in full strength.

In *Hyoscyamus* the symptoms are almost always of the type shown in Plate XXIX, fig. 1, and do not vary much with age. In all three hosts the older leaves tend to become necrotic and die away at the base of the plant.

In *Hyoscyamus* and tobacco there are well-marked preliminary symptoms which take the form of a "yellowing" or "clearing" of the veins (Plate XXVIII, fig. 3). They appear on the first two or three leaves which show infection after inoculation. A similar appearance is sometimes detectable in very young tomatoes at the same stage of infection. Preliminary symptoms occur in needle, rubbed and aphid-transmitted infection.

In some cases, particularly with *Hyoscyamus* and tobacco, death is caused by the virus. It generally occurs in the early stages of the disease and is much more common in the spring than in summer and autumn. Out of 49 positive results in *Hyoscyamus* fairly evenly distributed over 8 months, March to October 1931, there were 14 deaths, 11 of which occurred from inoculations made between March and June. Deaths of tomato have not been so common, but this may be due to the fact that most of the tomato inoculations were made later in the season of 1931, as there is one outstanding case for this year (1932) in which 35 out of a batch of 40 inoculated in April died in about a fortnight. Deaths of *Hyoscyamus* and tobacco during the spring of 1932 have so far been about as common as in the previous spring, but no comparative figures can yet be given.

*Filterability.* Experiments were carried out on the filterability of Hy. III. They were made using juice from tobacco, tomato and *Hyoscyamus* on different occasions, in the proportion of 1 gm. leaf material to 3 c.c. distilled water. Inoculation was made by needle into batches of tomato, *Hyoscyamus* and tobacco plants both immediately after filtration and after being kept for 24 hours.

The filtration was made through, first, a filter consisting of alternating layers of sand and paper pulp followed by Chamberland filter candles L 1 and L 3. The results were as Table I.

It is thus apparent that Hy. III will not pass an L 3 candle and hence appears to have larger particles than most of the known plant viruses, or else to have some special property by which it is absorbed on to the porcelain.



Table I.

*Filtration of Hy. III.*

Plants inoculated	Preliminary filter		L 1		L 3	
	1 hour	24 hours	1 hour	24 hours	1 hour	24 hours
6 <i>Hyoscyamus</i>	+	-	+	-	-	-
6 tomatoes	+	-	+	-	-	-
6 tobaccos	+	-	+	-	-	-

*Survival of the clarified juice.* It appeared from the above tests that Hy. III juice will not keep in a clarified state for 24 hours. Tests were also made to find out when it became uninfected. A series of tobacco plants were inoculated in batches of five at hourly periods with juice from tobacco filtered through an L 1 candle. After 24 hours two batches were inoculated with (a) the L 1 filtered juice, (b) unfiltered juice which had been kept as a leaf pulp.

Table II.

*Survival of clarified juice 1931.*

Plants inoculated	Period of keeping (hours)	Unfiltered juice (as leaf pulp)		L 1 filtered juice	
		Positive	Negative	Positive	Negative
5 tobacco	1	—	—	5	0
8 "	2	—	—	5	0
5 "	3	—	—	4	1
5 "	4	—	—	3	2
5 "	5	—	—	3	2
5 "	6	—	—	2	3
5 "	24	5	0	0	5

Similar results were obtained by inoculating batches of tomatoes at 2-hour periods for 8 hours. The suggestion from this experiment was that the clarified juice may last more than 8 hours, though it had lost a certain degree of its infective capacity in this time, and less than 24 hours. Unfortunately, as it was late in the season there were not large enough numbers of plants to repeat a long time experiment, but two further 24-hour experiments, using pulp filtered and L 1 juice, were made in September, both giving negative results.

These experiments were repeated in the spring of 1932 and the results did not agree with those of the previous autumn. Three experiments involving large numbers of plants were carried out, and in all cases the virus was infective after 24 hours. There seemed to be a slight diminution of virulence which can be observed in Table III.

Table III.

*Results on April 10th of inoculation from March 23rd, 1932.**Clarified juice from tobacco.*

Juice kept Individual tomato plant	1 hr.	2 hr.	3 hr.	4 hr.	5 hr.	6 hr.	7 hr.	8 hr.
1	x	0	x	x	x	✓	✓	✓
2	0	x	0	x	x	0	0	✓
3	x	x	x	0	0	0	0	✓
4	0	0	0	0	x	0	x	0
5	0	x	0	0	0	0	x	0

x dead plants.

0 dying plants (these all died during the week following the above observations).

✓ ordinary symptoms.

This experiment also demonstrates the lethal nature of the virus in the spring as compared with 55 inoculations made between August and October in the previous years in which no plants died—cf. p. 566. It has also been noted in three similar experiments that the longer the juice is kept the longer the period which elapses between inoculation and the appearance of the first symptoms. With juice kept for 24-hour periods the following results were obtained.

Table IV.

*Survival of clarified juice 1932. Juice from tomato.*

Juice kept	1 hr.	24 hr.	48 hr.	72 hr.
6 <i>Hyoscyamus</i>				
1	+	+	0	0
2	+		0	0
3	+	+	0	0
4	+	+	0	0
5	+		0	0
6	+	+	0	0

It is seen that there is a definite disparity in the survival periods of clarified juice between the autumn of 1931 and the early spring of 1932.

*Effect of heating.* Specimens of juice from infected tomato and *Hyoscyamus* were heated for 10 minutes in thin-walled tubes in a water bath kept constant at various temperatures. They were then inoculated into batches of plants with the following results:

*Juice from tomato.*

50° C.	5 <i>Hyoscyamus</i>	5 pos.
60° C.	5	5 neg.
70° C.	5	5 "
80° C.	5	5 "

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Similar results were obtained with juice from *Hyoscyamus* into tomato. Therefore Hy. III will survive a temperature of 50° C. but not of 60° C. or over.

*Intracellular inclusions.* Hy. III forms cell-inclusions in all three hosts; they are similar to aucuba and tobacco "X-bodies" but more loosely formed. In *Hyoscyamus* they are shapeless and often accompanied by spindle-shaped crystals. They have been seen in the hairs and epidermis (particularly along the veins of *Hyoscyamus* and in the hairs of tomato and tobacco).

### *Hy. II.*

*Host range.* Hy. II has not been as extensively tested for hosts as Hy. III, but is similar in its reactions as far as has been ascertained except that it causes no symptoms in tomato. In *Nicotiana glutinosa* it gives, in the young plant, faint "vein band" symptoms which rapidly disappear. The same range of potato varieties has been tested both by needle inoculation and aphid transmission with negative results. This is important because in its symptoms Hy. II resembles K. M. Smith's Crinkle Y [(5) (Plate XXX fig. 1)]. Crinkle, according to K. M. Smith, is composed of at least two viruses which occur together in nature: Crinkle X, a non-insect transmissible virus which causes ring-spot symptoms, and Crinkle Y which has a "vein band" type of symptoms and is aphid transmitted. In all the potato inoculations control half-tubers were kept, into which true Crinkle (Myatt's Ash Leaf) was inoculated and gave positive results. Healthy tubers (except Great Scot) and infected Crinkle stock were obtained by the kindness of Dr Kenneth M. Smith and Dr R. N. Salaman from the Potato Virus Research Station, Cambridge.

*Symptoms.* As has already been stated, the symptoms of Hy. II are primarily of the "vein band" type both in *Hyoscyamus* and in tobacco. In *Hyoscyamus* the "vein band" is a dark puckered area along the vein with very little interveinal mottle and no yellowing (Plate XXIX, fig. 3). In tobacco the symptoms are a typical "vein band" such as is described by K. M. Smith [(5), (Plate XXX, fig. 1)] and are quite indistinguishable from the symptoms of Crinkle Y in tobacco. Crinkle Y, however, causes a fine necrotic mottle in tomato, whereas Hy. II causes no symptoms. According to K. M. Smith [(6), *Hyoscyamus* crinkle causes "clearing of the vein within 7 days followed by characteristic darkening along the vein" but the photograph given on his Fig. 15, Plate 33, does not look like Hy. II in *Hyoscyamus*.

In *Hyoscyamus* the "vein bands" disappear on the older leaves, and

in the older plants on the young leaves which come up looking quite normal. The older leaves, however, always show a mottle though it may be very faint. In tobacco the vein bands generally persist throughout the life of the plant. The symptoms of Hy. II take longer to appear than those of Hy. III, generally 10 days to a fortnight, and preliminary symptoms of the Hy. III type (clearing of the veins) are only occasionally faintly discernible. The percentage of infection is not so regular as in Hy. III and varies between 70 and 100 per cent., thus larger batches of plants have to be used for each experiment.

*Filterability.* Parallel experiments on filterability to those on Hy. III were carried out on Hy. II and the results were practically the same except that it was rare to get full infection from the L 1 juice. It therefore agrees with Hy. III in being unable to pass an L 3 filter.

*Effect of heating.* Similar results were obtained as with Hy. III except that no trials were made at 50° C. The virus became inactive after immersion for 10 minutes at 60° C. and over.

*Intracellular inclusions.* The tissues of leaves, stems and hairs were examined for intracellular inclusions, but no definite abnormal condition could be found. In some cases the cytoplasm of the epidermis in the elongated cells above the veins seemed to be rather irregularly thickened and darker than in the normal condition.

#### *Insect transmission.*

##### *Hy. III.*

The first insect tested as a vector of these *Hyoscyamus* diseases was the flea beetle found occurring with them (*psylliodes hyoscyami* Linn.). The flea-beetles were cultured in the insectary and fed on infected *Hyoscyamus*; after a week's feeding they were removed to a series of young *Hyoscyamus* and tobacco seedlings, none of which showed any sign of disease after a suitable period had elapsed. This experiment was repeated several times and always with the same result.

As the diseases in some ways resemble potato Crinkle, the next insect tested was the well-known vector of potato Crinkle and leaf-roll, *Myzus persicae* Koch. The same routine was followed, but this time the disease was successfully transmitted in nearly every case.

Subsequent experiments show that it is freely transmissible by this insect vector between *Hyoscyamus* and tobacco and *Nicotiana glutinosa*, but cannot be taken to or from tomato except by direct inoculation. The preliminary and secondary symptoms caused by aphid transmission are

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precisely the same as the results of direct inoculation. Lethal characters are equally common and show about the same periodicity.

*Incubation period.* The experiments to find the length of time for which the aphid vector remains uninfective after feeding on the infected plant are, like the survival of the clarified juice experiments, divided into two sets, the first performed in late summer and autumn of 1931 and the second in the spring of 1932. Also, like the survival experiments, they show a definite disparity in the results between the two seasons. Unfortunately at the end of 1931 it was not possible, owing to lack of material, to obtain conclusive results, Table V being the average of only two experiments on longer periods (of 24 hours and over) and one on shorter periods. It must be pointed out that other experiments done as controls at the same time with 24 hours on the infected and five days on the uninfected plants gave 100 per cent. infection, so that it is certain that the virus, aphids and plants were in a normal condition as regards general possibilities. Six presumed infected aphids were placed on each seedling.

Table V.

*Experiments to test the period of time during which the vector of Hy. III remains uninfective.*

<i>Hyoscyamus</i> plants	1st feeding on infected plant (hours)	2nd feeding on healthy seedling (hours)	Total hours	Result	
				Positive	Negative
5	2	16	18	0	5
5	4	16	20	0	5
5	2	22	24	0	5
5	4	22	26	0	5
4	6	24	30	0	4
5	2	34	36	0	5
5	4	34	40	0	5
5	6	48	54	0	5
4	4	60	64	2	2
5	6	72	78	2	3
5	6	96	102	5	0

The results seemed to indicate that there was a definite "incubation" period of about 3 days, possibly more to obtain full infection. However, when the experiments were repeated three times in March and April of 1932, using larger batches and more varieties of plants (*Hyoscyamus*, tobacco and *N. glutinosa*, using 6 to 12 plants for each period tested) it was found that infection could be obtained in 30 hours, 6 hours feeding and 24 hours on the seedling. Both *Hyoscyamus* and tobacco were used as sources of infection and, in two experiments of 30 hours from start to

finish, gave 100 per cent. infection. The third gave a lower percentage infection throughout periods of 30, 54 and 78 hours and was possibly due to faulty feeding on the infected leaves.

### *Hy. II.*

The percentage of transference of *Hy. II* by *M. persicae* is even lower than the percentage from needle inoculation. In some experiments it is very high but this cannot be guaranteed and the conditions regulating it are unknown. Infection is carried between *Hyoscyamus*, tobacco and *N. glutinosa*. The symptoms are the same as those caused by needle inoculation or rubbing. Tomato is apparently immune to aphid infection as it is to needle inoculation.

*Incubation period.* Only one experiment has so far been done on the incubation period of *Hy. II*. It was made in March to April of 1932.

Table VI.

*Experiments to test the period of time during which the vector of Hy. II remains uninfective.*

Plants	1st feeding infected <i>Hyoscyamus</i> (hours)	2nd feeding healthy seedlings	Total hours	Result	
				Positive	Negative
6 <i>Hyoscyamus</i>	6	24	30	0	6
6 tobacco	6	24	30	0	6
6 <i>Hyoscyamus</i>	6	48	54	1	5
6 tobacco	6	48	54	0	6
6 <i>Hyoscyamus</i>	6	72	78	3	3
6 tobacco	6	72	78	4	2
6 <i>Hyoscyamus</i>	6	96	102	2	4
6 tobacco	6	96	102	3	3

This indicates that 30 hours is definitely insufficient; 2 days' feeding gives one positive result which may or may not be significant, and 3 and 4 days give about the same percentage. The suggestion is that there is an uninfected period in the insect of about 2 days, but more experiments will have to be made to verify this. Nothing is known as yet as to whether there is any seasonal difference.

## VIRUS DISEASES FROM FIELD B.

### *Hy. IV.*

*Host range.* *Hy. IV* was obtained from Field B in May 1931. It was first thought to be the same disease as was affecting plants in Field A, but the first inoculation into *Hyoscyamus* proved this to be wrong. It has been obtained on several occasions from Field B and varies only in

the strength of symptoms obtained at the first inoculation. Generally, after a few inoculations, they become identical, except in one case in which a strain brought from the field gave noticeably weaker symptoms even after passage through tomato. It gives 100 per cent. infection in *Hyoscyamus* and tomato. After passage through tomato it also gives positive results in tobacco. It has failed to cause any disease in the five varieties of potato, Arran Chief, Arran Victory, President, Epicure and Great Scot, which is one of its chief distinctions from Crinkle X which it otherwise resembles in symptoms (cf. K. M. Smith (5, 6)). Its infectivity throughout a much larger range of hosts is under examination.

*Symptoms.* In *Hyoscyamus* Hy. IV gives an all-over "pepper-and-salt" mottle slower in its appearance than Hy. III and not so vivid (Plate XXX, fig. 1). In some leaves this had a tendency to form rings, but these were never necrotic. The first few attempts to give the disease to tobacco failed altogether. In tomato it gave an "all-over" mottle similar to potato mosaic in tomato but, rather more of a "vein band" type (Plate XXIX, fig. 3). After passage through tomato it gave definite ring necrosis in *Hyoscyamus* (Plate XXIX, fig. 4) which, in the older plants, gave rise to a yellow mosaic, and also gave necrotic symptoms in tobacco (Plate XXIX, fig. 2). Similar symptoms in tobacco, though more of a mottle type with only occasional necrosis, were obtained without passage through tomato by inoculation from the original *Hyoscyamus* after about 3 months' growth. It is noteworthy that Crinkle X causes large necrotic lesions on the inoculated leaf [K. M. Smith (6) (Plate 33, fig. 16)] followed by ring-like lesions on the other leaves—whereas in Hy. IV the lesions formed by the virus after passage through tomato take the form of concentric rings on the inoculated leaves followed by ordinary mottle on the other leaves. It is not lethal in *Hyoscyamus* as is Crinkle X.

Hy. IV in tobacco is interesting in that symptoms appear first on the leaf next in order of growth to the one inoculated, and proceed in this order over the plant. In this way the older leaves become infective before the younger leaves which is directly opposite to the arrangement in many plant viruses in which the young leaves are the first to show infection. An experiment was arranged to determine whether the older leaves actually became infective before the younger ones:

A. A batch of six tobacco plants inoculated each on one leaf—(the oldest) from Hy. IV after passage through tomato. Symptoms did not show in A until the 11th day and then they appeared on the leaves (of the untouched pair) corresponding to those from which B and C were taken.

*B.* Four tobacco plants inoculated from the next oldest leaf of (two *A* plants) fourth day. Result—4 negative.

*B'.* Four tobacco plants inoculated from youngest leaf (two *A* plants)—fourth day. Result—4 negative.

*C.* Four tobacco plants inoculated from oldest leaf (two *A* plants)—ninth day. Result—2 positive, 2 negative.

*C'.* Four tobacco plants inoculated from youngest leaf (two *A* plants)—ninth day. Result—4 negative.

*Filterability.* Hy. IV can be filtered through an L 3 candle without any apparent diminution of infectivity.

*Survival of clarified juice.* The clarified juice of Hy. IV from tomato and *Hyoscyamus* is apparently unaffected by keeping for 48 hours and gives 100 per cent. infection.

*Effects of heating.* Heating in a water bath for 10 minutes at temperatures of 60°, 70° and 80° C. gave the following results.

Table VII.

*Effect of heating Hy. IV.*

Plants inoculated	60° C.	70° C.	80° C.
5 <i>Hyoscyamus</i>			
1	+	-	-
2	+	-	-
3	+	-	-
4	+	+	-
5	+	+	-

Similar results were obtained with tomato except that only one infection was obtained at 70° C. In one case an infection was obtained at 80° C. Hy. IV apparently survives 60° C. but its infectivity is impaired at 70° C. and almost entirely disappears at 80° C.

*Intracellular inclusions.* So far no traces of "X-bodies" or abnormal condition of the cells have been found in plants infected with Hy. IV.

*Insect transmission.* It has been found impossible to transmit Hy. IV by insects. *Myzus persicae*, *Macrosiphum gei* and *Thrips tabaci* have been tested as possible vectors without success. In this character it agrees with K. M. Smith's Crinkle X.

## DISCUSSION.

It is not proposed in this paper to draw any definite conclusions from the results so far obtained, but there are some problems which arise out of this work which are of interest. These may, for convenience, be tabulated as follows:

- (1) The relationship between Hy. II and Hy. III.



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(2) The relationship between Hy. II and Hy. IV as compared with Crinkle X and Y.

(3) The inability of *Myzus persicae* to transmit Hy. III to or from tomatoes.

(4) The disparity in results between experiments with Hy. III in autumn 1931 and spring 1932 and the possibility of a seasonal variation in the virus.

(1) It is fairly obvious that the relationship between Hy. II and Hy. III is very close as they both originated from the same inoculation and are very similar in their reaction. Both are non-filterable through an L 3 candle, both are killed at 60° C. and both are carried by *Myzus persicae* into the same range of hosts. The main problem is that Hy. III only appeared on one occasion and then only in tomato though, if it were present in the inoculum, it should also have given symptoms in *Hyoscyamus* as it is capable of completely masking symptoms of Hy. II. It is possible that Hy. II does exist in Hy. III plants and cannot be seen. This suggestion is supported by one experiment<sup>1</sup> in which Hy. III was inoculated from tobacco into *Hyoscyamus* and gave, in two cases, normal Hy. III symptoms and in one only symptoms of Hy. II. The fact that Hy. II causes no symptoms in tomato and has not yet been recovered from it, does not prove that it could not be present in the presence of Hy. III. This, however, does not explain the sudden appearance of Hy. III for which there would seem to be only three possible explanations.

(a) That it is an accidental infection which occurred after the first inoculation into *Hyoscyamus* and tomatoes and affected two of the latter with a virus which resembles the original inoculum exactly except for the violence of the symptoms, which is quite unique in some of its properties and which has never occurred before or since in the Rothamsted glass-houses. This is very unlikely.

(b) That it occurred in the one plant of Field A which happened to be used for this inoculation and in no other which was used previously or subsequently, and that, by some accident, it failed to produce symptoms in the *Hyoscyamus* and produced them only in two of the tomatoes.

(c) That it is a variant, mutant or dissociate from Hy. II induced by the sudden change in the conditions in which the virus was reared (from the field to hot-house condition) and the change in host plant.

The number of coincidences demanded by explanations (a) and (b) render these highly improbable and in view of recent researches on variation and dissociation of bacteria and lower fungi, explanation (c)

<sup>1</sup> This result has occurred again since going to press.

would seem to be the most probable. It is possible also that Hy. III itself is not simple and that another principle can be separated out by filtration (4) but this is still in process of investigation.

(2) Although Hy. II and Hy. IV do not form a natural group like Hy. II and Hy. III, the similarity between them and the group of diseases which forms potato Crinkle is very striking.

Table VII.

*Comparison between Hy. II, Crinkle Y, Hy. IV and Crinkle X.*

Hy. II	Crinkle Y	Hy. IV	Crinkle X
Vein band symptoms in <i>Hyoscyamus</i> and tobacco, followed by mottle in <i>Hyoscyamus</i>	Vein band symptoms in tobacco. Clearing of veins followed by vein-band symptoms in <i>Hyoscyamus</i>	Ring necrosis in <i>Hyoscyamus</i> and tobacco after passage through tomato: followed by mottle	Ring spot symptom in tobacco. Necrotic lesions followed by rings in <i>Hyoscyamus</i>
Carried by <i>M. persicae</i>	Carried by <i>M. persicae</i>	No insect vector	No insect vector
Will <i>not</i> go by needle or aphid into: Tomato, Arran Victory, Arran Chief, President, Epicure	Will go by needle and aphid into: Tomato, Arran Victory, Arran Chief, President, Epicure	Will <i>not</i> go by needle into: Arran Victory, Arran Chief, President, Epicure	Will go by needle into Arran Victory, Arran Chief, President, Epicure
Not filterable through L 3	L 3 filtration (no record)	Filterable through L 3	Filterable through L 3
Not found with Hy. IV in nature	Found in nature with Crinkle X and alone in Epicure	Not found in nature with Hy. II	Found in nature with Crinkle Y

Table VII is a comparison of various characters of Hy. II and Hy. IV, as investigated so far, with similar characters of Crinkle X and Y as described by K. M. Smith (5, 6). It can be seen that though they are very much alike in some of the symptoms, in insect transmission, in having the same host plant, and possibly in filterability, there are sufficient important differences to mark them as separate entities.

One of the important differences is their occurrence individually in separate fields so close together, as it is unlikely that diseases which originated in potato and afterwards infected the *Hyoscyamus* (or *vice versa*) should do so in such a way that they occur together in one crop and separately in the other.

At present the most likely hypothesis is that they constitute two parallel series of viruses occurring in separate hosts, of which the members of each set are not related to each other but to their parallels in the other series. Possibly the relationships are something in the nature of that between Hy. II and Hy. III which has a host difference in tomato.

(3) The fact that the disease (Hy. III) is not transmissible by *Myzus persicae* to or from tomato is interesting in view of the results obtained by Hoggan<sup>(3)</sup> on tobacco mosaic. In her experiments, tobacco mosaic is transmissible by *Myzus pseudosolani* from tomato to tobacco, but not from tobacco to tobacco or tomato. The suggested explanation is that "the aphid does not extract the virus from the tissues of the tobacco plant on which it feeds" (3). This, however, does not cover a case in which an insect which can be proved to be infective fails to transmit the disease, *i.e.* an aphid infected from tobacco and feeding on tomato. No explanation as yet presents itself to account for these facts.

(4) The discrepancy between results of experiments made in the autumn and the spring occurs in three aspects of the investigation. Data regarding the lethal symptoms come from spring and autumn of 1931 and spring 1932. The other two, namely the survival of the clarified juice and the existence or not of a so-called "incubation period" of the virus in the insect, only apply to the autumn of 1931 and the spring of 1932. It is obvious that further data are needed, at least results from August to October 1932, before any definite postulation can be made. The appearance of lethal characters in the spring suggests either a seasonal alteration of the virus or a seasonal increased susceptibility on the part of the plants. This might be due to the rapidity of growth of the spring plants, which is known to be favourable to the development of virus diseases. The other two facts cannot easily be dealt with in terms of the plant but, as only one season has elapsed, they might be due to some special condition peculiar to particular series of experiments. The three taken in conjunction do, however, suggest the possibility of a seasonal variation of the virus which is the explanation best agreeing with all the data so far accumulated, but this will require several seasons to establish.

#### SUMMARY.

1. The source and general character of three new virus diseases occurring in *Hyoscyamus* are described under the names of *Hyoscyamus virus* (Hy.) II, III and IV. They have a host range of various solanaceous plants not including any variety of potato.

2. Hy. II and III are non-filterable (through L 3) and are transmitted to and from all their hosts except tomato by the aphid *Myzus persicae*. Hy. III survives for a relatively short period as clarified juice. Hy. II and Hy. III have many characters in common and are probably closely related.



Fig. 1.



Fig. 2.



Fig. 3.





Fig. 1



Fig. 2



Fig. 3.



Fig. 4.



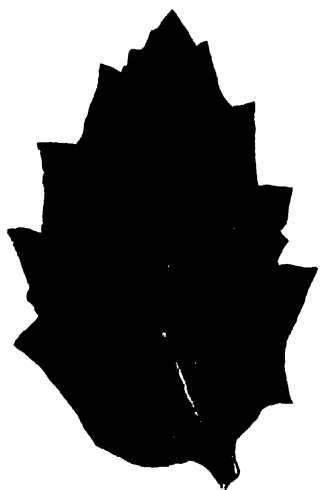


Fig. 1.



Fig. 2.



Fig. 3.

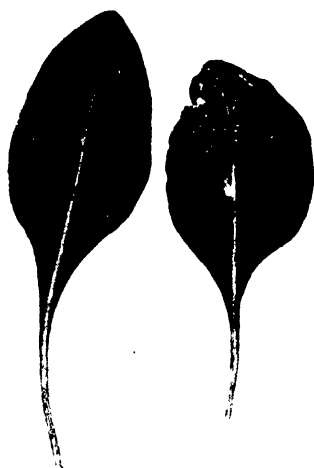


Fig. 4.





3. Hy. IV is a different type of virus, filterable through an L 3 candle and no insect vector has yet been found for it.

4. Problems arising from consideration of the data so far accumulated are submitted and discussed.

This work was carried out under the auspices of the Empire Marketing Board.

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### EXPLANATION OF PLATES XXVIII—XXX.

#### PLATE XXVIII.

- Fig. 1. Hy. II in *Hyoscyamus* plants brought in from field as winter buds. Infection was obtained from the two small plants, not from the large one. Height of large plant, about 3 feet.
- Fig. 2. Hy. III in tomato, two infected plants and one control of the same age. Height of medium plant, 19 cm.
- Fig. 3. Young *Hyoscyamus* plant showing preliminary symptoms ("Clearing of the veins") of Hy. III.

#### PLATE XXIX.

- Fig. 1. Hy. III in *Hyoscyamus* typical late symptoms; compare with similar type of symptom in tobacco.
- Fig. 2. Hy. III in tobacco, typical late symptoms. Length of leaf, 15 and 18 cm.
- Fig. 3. Hy. II in tobacco, compare with Fig. 4. Length of leaf, 21 cm.
- Fig. 4. Hy. II in *Hyoscyamus*; note the puckered bands along the sides of the vein. Length of leaf, 15 cm.

#### PLATE XXX.

- Fig. 1. Hy. IV in *Hyoscyamus*, "pepper and salt" mottle. Length of leaf, 14 cm.
- Fig. 2. Hy. IV in tobacco, after passage through tomato. Length of leaf, 23 cm.
- Fig. 3. Hy. IV in tomato.
- Fig. 4. Hy. IV in *Hyoscyamus*, after passage through tomato. Length of leaves, 8–8½ cm.

(Received April 18th, 1932.)

# THE APPEARANCE AND BEHAVIOUR OF PIGMENT IN THE SUFFOLK BREED OF SHEEP

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(With Plates XXXI—XXXIII and 5 Text-figures.)

## INTRODUCTION.

THE colour changes exhibited by the Herdwick and Suffolk, during the transition of the birthcoat to that of the adult, are a direct expression of metabolic function. Their definite correlation with the age of the individual is very striking, and it was thought that an investigation of the character of the fleece during the period of colour change would throw further light on the pigmentary system of the sheep. In 1927 Nichols published a paper on "The Occurrence of Dark Fibres in the Suffolk Fleece with particular Reference to the Birthcoat of the Lamb," and for this reason it was decided to select the Suffolk, in preference to the Herdwick for further research.

The Suffolk originated from a cross between the Old Norfolk Horn and the Southdown, and for the last seventy years has been recognised as a standard breed of sheep.

In addition to its excellent mutton qualities, it possesses an extremely desirable fleece of fine white wool, which in common with that of other Down breeds approaches more nearly to the Merino fleece in fineness than to those of many other British breeds of sheep. The Suffolk is presently greatly in demand for export purposes and its use for crossing with other breeds is being greatly extended. For example, this is especially the case in South Africa where experimental crossing with Merino and Native sheep, with a view to producing a "dual purpose" sheep, is being undertaken. The character of the cross-bred fleece is in these cases a matter of primary importance, and it is essential that the breeder should understand the nature of the parent fleece; in this connection the behaviour of the pigmentary system is of the greatest importance.

In the adult, the face and legs are uniformly black whilst the body is covered with fine white wool and a small quantity of fine white wool in the

form of a tuft sometimes occurs on the forehead. The birthcoat of the lamb is, however, quite different; it may be of a uniform dun colour, speckled black and white, or pure black.

#### EXPERIMENTAL MATERIAL.

Five lambs, approximately eight weeks old, were selected from a flock of pedigreed Suffolks for observation. In a preliminary study a brief analysis of samples of wool from each parent was made. In each case a very small percentage of both black and white kemp was present; a few completely pigmented wool fibres occurred and there were several white wool fibres with coloured tips; in other respects the wool was distinctly clean and very fine.

Eight samples were taken from each lamb at approximately monthly intervals during a period of one year, June 1929 to May 1930, and carefully analysed. The sampling areas were:

- (1) Median dorsal neck.
- (2) Median ventral throat.
- (3) Left shoulder.
- (4) Left flank.
- (5) Left rump.
- (6) Britch.
- (7) Belly.
- (8) Left thigh.

For all practical purposes it was found that three samples (1, 3, 6) from each would have been sufficient.

The practice followed was to make general observations on the sample, always from an area about 2 cm. square, comprising about 3500 fibres and then to remove from it at random 100 fibres. The special interest in this study was found in the microscopic examination and analysis of the fibre types. Nichols defined three such types, as follows:

- (1) Wool fibres with pigmented tips.
- (2) Kemp.
- (3) Finely medullated, coloured, coarser intermediate fibres.

After the first two months and for the remainder of the year, all the kemp fibres in the samples were deliberately picked out together with every other obviously coloured fibre, and a number of completely white fibres. The gradual decrease in the proportion of coloured fibres made such a selection desirable. For microscopic examination the fibres were washed in ether (the whole sample had previously been washed in warm benzene and in water) and then mounts were made in glyco-alcohol, euparal and

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cedarwood oil. Strong sulphuric acid, followed by gentle heating, was used for fibre disintegration work. As each set of samples was analysed the results were summarised and compared. (See Appendix Charts 1-5.)

From the outset, one lamb showed considerably less pigmentation than the others and a year later was possessed of by far the cleanest fleece. If this may be regarded as typical, the significance of an early analysis of the lamb's coat in predicting the colour character of the fleece of the adult is obvious. In March 1930 the britch sample from this same lamb included a patch of coloured fibres, but apparently such promiscuous spotting is inevitable in sheep which genetically carry colour factors. The shoulder region is not a good colour indicator since it clears very rapidly and even in sheep from commercial Suffolk flocks has only a very small percentage of coloured fibres.

### COLOUR IN THE VARIOUS FIBRE TYPES.

The three types of fibres occur in both lamb and adult fleeces, although the fibres of type 3, finely medullated, coloured, and somewhat coarse intermediate fibres, are reduced to a minimum in the adult. In addition, completely coloured true wool fibres which are not present in the birth-coat are found in the adult fleece. Apart from the successive generations of coloured kemp fibres described by Nichols which occur in the fleece as a whole, it was found that kemps showing only a small extent of pigmentation were specially prevalent in the axillary regions and to a less extent on the belly. It was on these regions also that the apparently white kemps occurred. Throughout the life of the Suffolk the ventral portion of the fleece is always darker than elsewhere and there is a greater preponderance of the pigmented finely medullated type of fibre on the belly.

#### *Type I—Kemp.*

The kemp series exhibited extreme variation both in form and colour (Plate XXXI). They were found to be sometimes black-tipped or entirely white, black, brown, fawn or red. They might be stiff, straight and short, short and wavy, or irregularly crimped, or in some cases might show elbowing.

#### *Type II—Wool.*

The wool fibres in the lamb coat were all colour-tipped, the coloured distal portion sometimes extending practically the whole length of the fibre (Plate XXXI). Often these coloured tips, which are easily broken off, were found lying free in the sample, but it was not difficult to distinguish them from coloured kemps.

*Type III—Medullated Fibres (Intermediates).*

These fibres exhibited extreme variation (Plate XXXI). In some instances they were long, coloured (black, red, etc.) hair-like, and often exceeded the length of the wool fibres. In others they were heterotypic in colour, being fawn distally and grey proximally, or black distally and white proximally or showed intermittent banding. Some were even heterotypic in form, strongly resembling, save in colour, the heterotype of the Merino (Duerden and Seale, 1927) being stiff and straight distally, and finely crimped and woolly, proximally. These fibres varied tremendously in diameter.

During the first few months there is a gradual decrease in the quantity of colour tipped wool and coloured fibres generally, and the character of the adult fleece becomes more and more defined by the increase in the fine white wool. Nichol's work indicates that the follicles of the intermediate coloured fibre type give rise to wool fibres. This is supported by the presence and appearance of colour-tipped wool fibres in the fleece throughout the life of the adult, the colour tipping being due to the fact that the cells producing such fibres are potentially pigment producing.

In the samples examined, every conceivable grade of colour was seen in the pigmented fibres although those of the kemp series were usually red, black or "colourless" (*i.e.* to the naked eye). There was extreme variation in the pigmentation of the wool and bipartites<sup>1</sup> and banded fibres were often found. The significance of the occurrence of colour-tipped white kemp fibres is discussed later.

## MICROSCOPIC OBSERVATIONS.

The minute details of deposition and disposition of pigment in the fibres was carefully studied and it was soon evident that no apparent genetic significance could be attached to these characters, since both the morphology of pigment granules and their arrangement in a fibre seemed to be fortuitous. It is not yet known at what stage the differentiation of fibre cells into cuticle, cortex and medulla takes place and what it is that determines that either cortex or medulla or both shall contain pigment. In the Suffolk it would appear to be a matter of chance. In other animals, however, where self-colour is a genetic and fixed character, the inclusion and manner of deposition of pigment in the cortex only or the medulla only, or both, are within limits, predetermined, and it is this maintenance of pigment deposition and disposition which preserves breed coloration, so that the Aberdeen Angus, for example, is black, the Jersey

<sup>1</sup> Fibres approximately half-coloured and half-white, at the time of examination.

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is a fawn, and, possibly most static of all, the Suffolk horse is chestnut. Various coloured mice illustrate this point very clearly. Brown, black, grey and fawn mice owe their apparently different colours only to the variation in quantity and disposition of the pigment in the fibres.

The fortuitous manner in which pigment is included in the Suffolk fibre explains why any single fibre may exhibit profound colour differences along its length. It is significant that not in any wool fibres examined did pigment occur in the cuticle, although Hausman (1928) suggests that occasionally, although very rarely, the cuticular scales of infrahominid mammals may be tinted as though stained by diffuse pigment, and he records a sparse occurrence of pigment granules in the transparent cuticle of Chinese and Japanese human hairs. It is obvious that the structure of the cuticle in wool fibres, at least, does not permit of pigmentation. During the study of the histology of the developing Suffolk fibre which was carried out in connection with this work (see Plate XXXII *a*) particular attention was paid to this point. Papillal cells which later gave rise to cuticular scales were observed to contain pigment. Extended observations showed, however, that as these cells moved upwards in the follicle the pigment might migrate into the cortex or be pressed out into the intercellular spaces or else might migrate to the dermis, where it was phagocytosed. The thin leaf-like structure of a cuticular scale conclusively shows that pigment inclusions are a physical impossibility (Plate XXXIII).

Pigment granules may be so small as to be unmeasurable, so fine as to be invisible, save as a diffuse stain, or so large as to obscure cell structure. They may occur in such quantity as to deform the natural profile of a fibre, or be so few in number as to leave the fibre colourless. When pigment appears to assume a definite regular shape the cause may often be attributed to the structural restrictions of the cells of the fibre, and in the Suffolk where heavily pigmented fibres appear to contain spindle-shaped pigment masses (Nichols) in reality the spindle form is defined by the spindle-shaped cortical cells (Plate XXXIII). On the other hand, many fibres do appear to exhibit a definite arrangement of pigment granules and a linear grouping is often found. Such an arrangement is, however, only mechanical and illustrative of the compression and subsequent elongation of the cortical fibre cells as they are pushed upwards from the apex of the papilla, preceding keratinisation.

When diffuse pigment is produced in small quantities it assumes a characteristic streak-like appearance, due to the fact that during shrinkage of the fibre cells just at the time when keratinisation occurs, the diffuse nature of the pigment allows it to seep through and flow into the

cellular interspaces (Plate XXXIII). In this study hundreds of fibres were disintegrated in hot concentrated sulphuric acid, and the behaviour of the pigment granules studied. Diffuse pigment under these conditions is readily soluble, whilst granular pigment remains insoluble.

The presence of pigment aggregations in the fibre in the form of apposed bell-shaped masses was at first somewhat puzzling (Plate XXXIII). They occur in practically every pigmented fibre, but are best seen in lightly pigmented fibres where their outline is not obscured by quantities of pigment inclusions. Later investigations of the histology of the skin and its developing follicles explained their appearance. These bell-shaped masses are formed by the aggregation of pigment granules over the nucleus of the apical cells of the papillae and their maintenance in such a position suggests that they are protective in function. These nuclear caps, single or double, have also been recorded in man (Percival and Stewart), cattle (Esskuchen), pigs (Teodoreanu) and horses (Ludford).

During development of the fibre many pigmentogenic cells pass up from the bulb unchanged, and keratinisation, whilst destroying the protoplasmic contents of the cells does not affect the pigment inclusions, so that the original cell outline is preserved. It is not unusual to find series of these undifferentiated pigment cells lying along the length of a fibre, exactly similar in appearance to the basal dopa-positive cells of the epidermis (Plate XXXIII).

Observations of dendritic pigment cells *in vitro* demonstrates quite conclusively the extraordinary activity and behaviour of pigment in the living cell and offers sufficient explanation for the extreme variation in deposition and disposition which is seen in the keratinised wool fibre.

#### DISCUSSION.

The definition and assumption of the adult fleece depends on a restriction of the activities of the pigmentary system. The genetic constitution of the Suffolk apparently ensures that in the head and legs the inherited potentiality for pigment production shall always find expression, but that elsewhere the pigmentary system shall be more or less quiescent, responding only to definite additional stimuli. The phenomenon of self-colour in the young followed by an entirely different appearance in the adult is by no means restricted to sheep and is a not uncommon occurrence throughout the animal kingdom. It may be seen in birds, *e.g.* Gannet; rabbits, *e.g.* Argenté; Llamas, etc. The exhibition of a colour change during the first period of independent existence is naturally controlled by the genetic stability or versatility of the pigmentary system,



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and it may be assumed that where a retrogressive colour change does take place the pigmentary system fails to function because the chemical environment is unsuitable. That this environment could be controlled is probable. The colour change is correlated with general body development and it is reasonable to postulate that just as the maintenance of an axolotl in a certain environment prevents its assumption of adult characters, so would the retardation of development in the Suffolk prolong the retention of a wholly pigmented fleece.

A lack of comprehensive data makes it impossible to define precisely the nature of the interference which modifies the functioning of the pigmentary system, but whatever the cause, the immediate inhibitor is probably the inertia of the nucleus in so far as the production of the oxidising enzyme is concerned. Support for this theory is found in the fact that since scattered coloured fibres develop sporadically in the fleece the production of dihydroxyphenylalanine from tyrosin and the circulation of this substrate in the blood must be a constant occurrence. The appearance of colour tipped white wool fibres indicates that the intensive cellular activity which accompanies the initiation of fibre growth is correlated with pigment production. The variation in the length of the coloured tip, however, is more difficult to explain, but it must bear some relation to the strength of the original stimulus. Although we may accept the fact that dopa is always circulating in the blood a variation in quantity must be considered, and when only small amounts are present it is possible that not every cell nor every follicle receives its quota. This offers a further explanation for the occurrence of scattered pigment granules in practically colourless fibres, and for pigment being found along one side of a fibre only, in caps, in dense masses, etc. Since the characteristic metabolism of the body determines the quantity of protein which is available for melanin synthesis, it is possible that in those animals where adult and birthcoat coloration differs, with development, the available melanin precursor is diminished. This indicates an interesting line for investigation.

All sheep, even within a breed, show great variation in the time at which the pigmentary system begins to function, but, generally speaking, pigment deposition commences about the end of the first month of intra-uterine life. In the Suffolk it has been observed before the end of the fourth week in minute quantities round the lips, and as a rule the head and hoofs show pigmentation before the body generally.

It cannot be said that the Suffolk has ever been shown to exhibit the Schultz reaction, and the presence of colour-tipped white wool fibres is in no way connected with the Schultz reaction. The regeneration of coloured

or colour-tipped fibres in place of white ones after injury, after maggot attack involving shedding of the fleece, or shaving, is due to the increased cellular activity of the cutaneous system inducing a response on the part of the pigmentary system.

The Suffolk, in point of fact, exhibits precisely the reverse conditions of those that obtain in the Himalayan rabbit and in it, temperature cannot be considered even one of the many controlling factors in the activities of the pigmentary system. Nichols' postulation of "thresholds of irritation (minimal levels of metabolism for effective action, which can be affected by temperature)" is therefore not acceptable.

The browning of the hairy covering of the face which is sometimes seen in the Suffolk ewe after lambing is due to debility and therefore decreased metabolic activity and is somewhat comparable with the decoloration which accompanies age.

The microscopic survey of the fibres of the Suffolk has demonstrated within one breed, a variability of activity on the part of the pigmentary system, such as is almost inconceivable. It has proved of great value in estimating the nature of other colour phenomena, and has often prevented rash assumptions and inaccurate interpretations.

The phenomenon of "Colour Banding in the Fleece" is one case in point, and since the completion of the Suffolk work, it has been possible to suggest an explanation of the phenomenon which before seemed incapable of interpretation. It does not appear to be extravagant to state that given the suitable environment any coloured sheep would produce a banded fleece, for banding may be defined as a "somatic manifestation of a cryptomeric character," using the term character in its widest sense. The expression of the character apparently depends on an environmental variation, and in so far as the genetic significance of banding is concerned, it is not so much the character for banding which is heritable as a tendency by which variation in protein metabolism affects the pigmentary system of one animal more than another. Remembering the occurrence of tipped and banded fibres in the Suffolk, it would appear to be only a matter of degree. Every variety of wool is possibly a banded wool, only the banding is restricted and therefore obscured.

#### SUMMARY.

The results of a microscopic study made of the colour transition shown by the fleece of the Suffolk from birth to maturity are given. It is demonstrated that the physical structure of the fibre cuticle obviates the possibility of pigment inclusions.

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An explanation for the sporadic occurrence of coloured fibres in an otherwise white fleece is attempted, and it is pointed out that, however else the pigmentary system of the Suffolk sheep may be stimulated, thresholds of irritation conditioned by temperature need not be considered as possible stimuli.

### ACKNOWLEDGMENTS.

The writer wishes to thank Prof. F. A. E. Crew, Director of the Institute of Animal Genetics, University of Edinburgh, for the scientific hospitality she has received in his department. She acknowledges gratefully the interest Mr Wm. C. Miller has always taken in this research, and finally, her best thanks are due to Mr J. P. Ross Taylor, Mungoswells, Duns, who has so generously provided all the material necessary for this study.

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### APPENDIX CHARTS 1-5.

To illustrate the distribution of fibre types in Suffolk lambs I, II, III, IV, V. The crosses refer to the occurrence in the respective areas of the fibre types which they are opposite. Column R records the occurrence of red coloration; the figures refer to areas.

### EXPLANATION OF PLATES XXXI—XXXIII.

#### PLATE XXXI.

Fibre types.

#### PLATE XXXII.

(a) Micro-photograph of skin section cut longitudinally through the fibre follicles from the shoulder of a twelve weeks old Suffolk foetus.  $\times 100$  (circa).

(b) Micro-photograph of a section through the skin of the nose of a foetal lamb  $\pm 4$  weeks, illustrating the pigmentogenic cells of the Malpighian layer.  $\times 250$  (circa).

#### PLATE XXXIII.

Types of pigmentation in fibres from Suffolk wool.

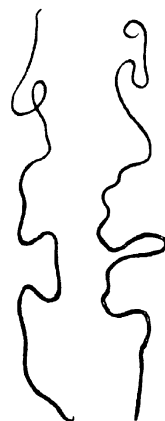
(Received December 9th, 1931.)



Colour-tipped Wool



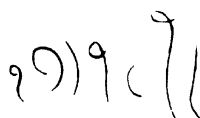
Banded Wool



Bi-partite Wool



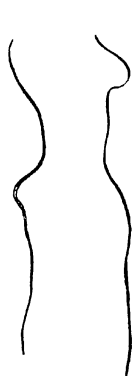
Black Kemp



Red Kemp



Colour-tipped white Kemp



i Banded



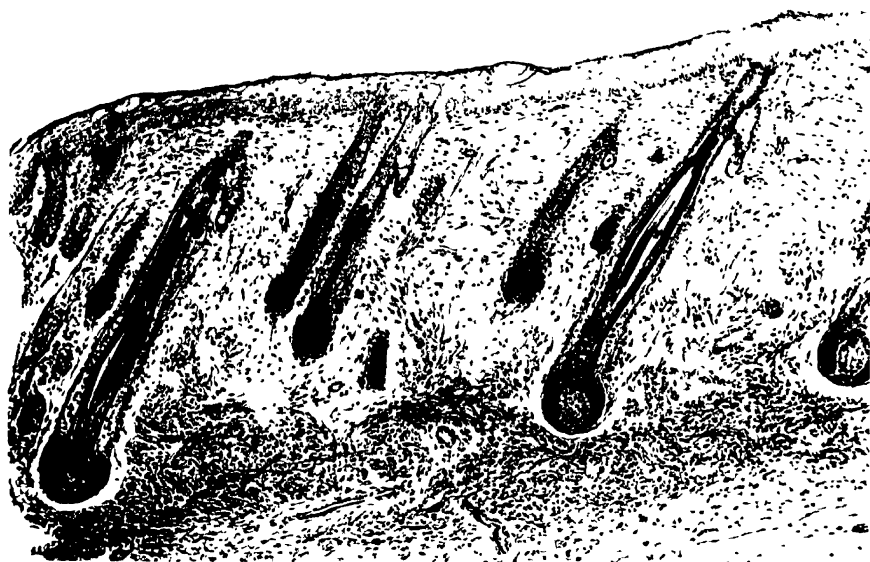
ii Coloured



iii Bi-partite

INTERMEDIATES



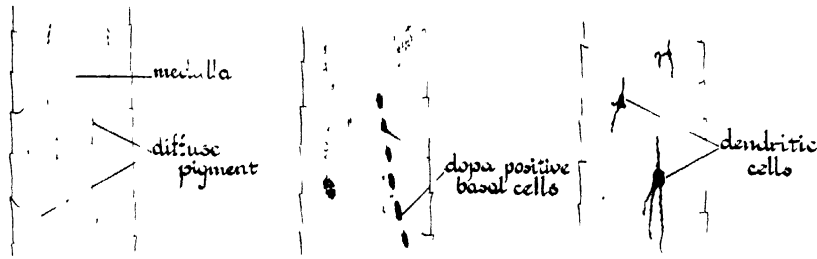


b

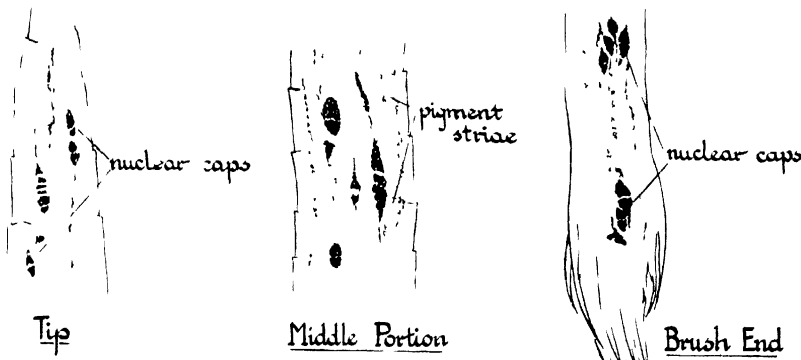




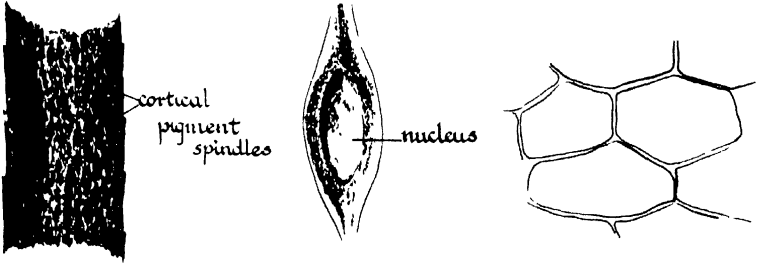




Portions of Fibres showing Diffuse and Granular Pigment [x 100 circa]



Lightly Pigmented Fibre [x 100 circa]



Single Cortical Cell x 1000

Heavily Pigmented Fibre  
[x 400 circa]

Colourless, Flat, Cuticular Scales  
[x 1000]



AREAS	MAY JUNE								R		JULY AUGUST								R
	1	2	3	4	5	6	7	8			1	2	3	4	5	6	7	8	
Wool: Tipped	x	x	x		x				8		x	x	x		x	x	1		
Coloured																	2		
Banded																	4		
Kemp: Black	x		x	x				x	x		x	x	x		x	x	5		
Red		x															6		
Colourless														x		x			
Black-tipped white																			
Intermediates																			
Coloured	x				x			x	x						x				
Hetero-coloured				x				x			x				x				
Banded	x					x			x										
Bi-partites																			
	SEPTEMBER OCTOBER								1		NOVEMBER DECEMBER								1
Wool: Tipped	x	x	x	x	x	x	x	8			x	x	x		x	x	x	2	
Coloured										x							3		
Banded																	5		
Kemp: Black	x	x			x	x	x	x									6		
Red											x	x	x	x		x	8		
Colourless								x											
Black-tipped white								x											
Intermediates																			
Coloured								x			x					x			
Hetero-coloured																x			
Banded																			
Bi-partite															x				
	JANUARY FEBRUARY								1		MARCH APRIL								6
Wool: Tipped	x	x	x	x	x	x	x	3			x	x	x	x	x	x	x	x	
Coloured								8											
Banded																			
Kemp: Black	x	x	x			x	x	x		x	x			x	x				
Red	x		x					x											
Colourless														x					
Black-tipped white															x				
Intermediates																			
Coloured								x											
Hetero-coloured									x										
Banded	x																		
Bi-partites																			

**APPENDIX CHART 1. LAMB I**

AREAS		MAY JUNE										JULY AUGUST									
		1	2	3	4	5	6	7	8			R	1	2	3	4	5	6	7	8	R
Wool:	Tipped	x		x	x		x					x	x	x		x	x	x	4		
	Coloured																		5		
	Banded																		8		
Kemp:	Black	x	x	x	x							x	x		x		x	x			
	Red																				
	Colourless																x				
	Black-tipped white								x												
Intermediates:																					
	Coloured	.				x		x		x			x		x			x			
	Hetero-coloured			x											x						
	Banded							x							x	x					
	Bi-partites																				
		SEPTEMBER OCTOBER										NOVEMBER DECEMBER									
Wool:	Tipped	x	x	x	x	x	x	x	x	5		x	x	x	x		x	x	1		
	Coloured									8		x							2		
	Banded																		5		
Kemp:	Black	x		x	x	x			x			x	x	x	x	x	x	x	7		
	Red																				
	Colourless								x							x					
	Black-tipped white								x												
Intermediates:																					
	Coloured							x				x						x			
	Hetero-coloured	x										x		x				x			
	Banded																				
	Bi-partite															x					
		JANUARY FEBRUARY										MARCH APRIL									
Wool:	Tipped	x	x	x	x	x	x	x	x			x	x	x	x	x	x	x			
	Coloured																				
	Banded																				
Kemp:	Black	x	x	x		x	x	x	x			x	x	x	x	x	x	x			
	Red																				
	Colourless															x					
	Black-tipped white															x		x	x		
Intermediates:																					
	Coloured								x			x									
	Hetero-coloured							x													
	Banded									x											
	Bi-partites	x																			

APPENDIX CHART 2. LAMB II

AREAS		MAY JUNE										JULY AUGUST								
		1	2	3	4	5	6	7	8			R	1	2	3	4	5	6	7	8
Wool:	Tipped	x	x							1		x	x	x	x	x		x	x	5
	Coloured																			
	Banded																			
Kemp:	Black	x	x	x	x	x						x	x			x		x		
	Red																			
	Colourless																			
	Black-tipped white																			
Intermediates:																				
	Coloured	x				x		x				x		x		x		x	x	
	Hetero-coloured				x			x									x		x	
	Banded					x														
	Bi-partites																			
		SEPTEMBER OCTOBER										NOVEMBER DECEMBER								
Wool:	Tipped	x		x	x	x	x	x	x	5		x	x	x	x	x	x		1	
	Coloured									6									5	
	Banded									8									6	
Kemp	Black	x	x		x				x			x				x	x	x	7	
	Red											x							8	
	Colourless				x															
	Black-tipped white																			
Intermediates																				
	Coloured							x				x				x	x		x	
	Hetero-coloured		x																	
	Banded																	x		
	Bi-partites																			
		JANUARY FEBRUARY										MARCH APRIL								
Wool:	Tipped	x	x	x	x	x		x	x	5		x	x	x	x	x	x		4	
	Coloured									7									6	
	Banded																			
Kemp:	Black	x	x											x	x	x				
	Red					x														
	Colourless								x											
	Black-tipped white																	x		
Intermediates																				
	Coloured																		x	
	Hetero-coloured																			
	Banded																			
	Bi-partites																			

APPENDIX CHART 3. LAMB III

AREAS		MAY JUNE								R			JULY AUGUST								R
		1	2	3	4	5	6	7	8				1	2	3	4	5	6	7	8	
Wool:	Tipped	x	x	x	x		x			4			x	x			x	x		1	
	Coloured									8										2	
	Banded																			3	
Kemp:	Black	x	x	x				x	x				x	x			x	x		6	
	Red																			7	
	Colourless																x				
	Black-tipped white								x												
Intermediates:																					
	Coloured	x			x				x				x		x		x	x			
	Hetero-coloured			x				x					x		x		x	x			
	Banded	x	x		x								x		x		x	x			
	Bi-partites																x				
		SEPTEMBER OCTOBER											NOVEMBER DECEMBER								
Wool:	Tipped		x	x		x		x		5				x		x	x			4	
	Coloured									7				x						7	
	Banded	x		x																8	
Kemp	Black	x	x	x	x	x	x	x	x				x			x	x	x	x		
	Red						x														
	Colourless																				
	Black-tipped white																				
Intermediates																					
	Coloured	x	x				x	x					x		x		x	x			
	Hetero-coloured													x		x		x	x		
	Banded	x																			
	Bi-partites	x			x	x															
		JANUARY FEBRUARY											MARCH APRIL								
Wool	Tipped		x	x		x	x	x		2			x	x	x	x	x	x		5	
	Coloured													x						6	
	Banded																			7	
																				8	
Kemp	Black	x	x	x		x	x		x				x	x	x				x		
	Red																		x		
	Colourless																				
	Black-tipped white								x												
Intermediates																					
	Coloured		x												x						
	Hetero-coloured	x		x		x															
	Banded								x						x	x					
	Bi-partites		x		x									x			x	x	x		

APPENDIX CHART 4. LAMB IV





## THE EFFECT OF *COLPIDIUM* ON AMMONIA PRODUCTION BY SOIL BACTERIA

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(With 8 Text-figures.)

### INTRODUCTION.

THE problem of the effect of protozoa on bacterial activities is by no means a simple one. One aspect of this effect, namely the feeding or "phagocytic" action of protozoa, has been studied for a long time. It is well known that many species of protozoa derive most, if not all, of their nutriment from bacteria, and will reduce the numbers of bacteria in their surroundings by feeding upon them. It is when the chemical activities of the bacteria come to be considered that complications arise.

It might be supposed at first sight that, since protozoa will reduce the numbers of bacteria in a culture, the chemical activities of that culture will necessarily be reduced in their presence.

That this is not so in every case was shown by Cutler and Bal in the case of nitrogen fixation by *Azotobacter*, where an increased amount of nitrogen was found to be fixed in presence of protozoa (1).

The idea that reduction of bacterial numbers implies a decrease in the amount of a given product formed by the bacteria proceeds from the supposition, widely held, that the metabolic activity of a culture is simply proportional to the numbers of bacteria in that culture, that is that 2000 million bacteria will produce ten times as much of any given product as 200 million bacteria under the same conditions. This is tacitly assumed to be the case in much work, especially on soil bacteria, where the amount of some metabolic product is used as a measure of bacterial growth. In all such work the amount of a given product produced per individual organism is held to be constant, and independent of the numbers of bacteria present.

In a previous paper on ammonia production by a soil bacterium (2), a case was described where this is not so, the amount of ammonia produced per individual organism being found to be inversely proportional to the numbers of bacteria present. It followed from this that the actual

amount of ammonia produced increased with the bacterial numbers up to a certain point, but numbers of bacteria above this "optimum" level produced actually less ammonia. The possible bearing of this relation between bacterial numbers and activity on the effects of the presence of protozoa in a bacterial culture was indicated by some preliminary experiments with a soil amoeba (*Hartmanella hyalina*). In these experiments the amoebae were found to reduce the numbers of bacteria in sand cultures (compared with control cultures in which no amoebae were present), in the majority of cases, while the ammonia production was found to be greater in the cultures containing amoebae. As a partial explanation of these results, it was suggested that the amoebae reduced the bacterial numbers from a value above the "optimum" level to one nearer to it, thus automatically increasing the amount of ammonia produced.

To obtain further data on this point the experiments described in this paper were carried out, in which liquid media were used, and the amounts of ammonia (and in some cases of carbon dioxide) produced by bacteria in the presence and absence of protozoa were noted. The species of protozoon used in these experiments was a ciliate, a species of *Colpidium*; the particular strain employed resembled very closely the strain of *C. colpoda* used by Cutler and Crump(2), and obtained by them from Peters. The dimensions of an average organism ( $50\mu \times 25\mu$ ) agreed in both cases, but the undulating membrane round the mouth, which was easily seen in the Peters strain, is hardly visible in the one here described.

*Colpidium* was chosen for this work firstly because it was relatively easy to obtain and grow in culture, and secondly because it (or a related form) had previously been studied in this department(1, 2), and many of its properties were therefore well known.

In every experiment a culture containing *Colpidium* in company with a known bacterial flora was compared against a culture of the same bacteria alone, under identical conditions; the results obtained from the two series of cultures are, therefore, directly comparable.

In the choice of a medium two factors had to be considered, the suitability of the medium for the growth of *Colpidium*, and the presence of some nitrogenous compound to act as a source of ammonia.

The stock cultures of *Colpidium* were grown on soil extract, so the first series of experiments described here were carried out in a solution of peptone in soil extract. This medium, while it was suitable for the growth of *Colpidium*, was not considered satisfactory, as its composition was unknown and could not be exactly repeated at each making.

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As a result of a series of trials with different simple nitrogenous substances, a medium containing alanine in a mineral salt solution was found to give satisfactory growth of *Colpidium*. This medium was of known composition and could be exactly repeated, and it was used in the second series of experiments.

It should be noticed that the bacterial numbers given throughout this work represent total counts, made with a counting chamber. It was felt that this method was to be preferred to a plate count, as there is no evidence that bacteria which are no longer capable of developing on the plate have ceased to show any metabolic activity.

### METHODS. (GENERAL.)

In each experiment a pair of flasks, containing a known volume of liquid medium, was used. They were inoculated from parent cultures grown on soil extract, one flask of each pair with *Colpidia* and their accompanying bacteria, and the other with the same bacteria alone. The flasks were kept at room temperature, and the bacteria and protozoa in them counted daily. The ammonia in each culture was determined at intervals, and in some experiments a stream of air was passed through the cultures, and the carbon dioxide given off recorded.

The stock of *Colpidium* was obtained in April 1930, and was maintained on soil extract, made from farmyard manured soil. The early subcultures from the original stock culture were enriched with the bacterium YB (this enrichment has been repeated at every sub-culturing), and later attempts were made to obtain the *Colpidia* in pure culture with YB, by washing and inoculating the washed organisms into suspensions of YB in soil extract. These attempts, however, were not wholly successful; it was found impossible to eliminate one strain of bacteria which accompanied the *Colpidia* (referred to as strain CO). The *Colpidium* cultures used in this series of experiments contained, therefore, two strains of bacteria, YB and CO, the characteristics of which were as follows:

YB rods,  $1.6-2.0\mu \times 0.8\mu$ , occasionally in pairs, non-motile, do not form spores, gram positive (weak); agar streak—growth abundant, smooth, yellow, opaque, medium unchanged; colonies on Thornton's medium—round umbonate, white or pale yellow, smooth shining, edge entire; liquefies gelatine slowly, produces acid on dextrose only (out of all sugars tested), no diastatic action on starch, reduces nitrates to nitrites; it will produce nitrite from ammonium phosphate in presence of dextrose or glycerine (there is evidence that this property has been only recently acquired); optimum temperature about  $25^{\circ}\text{C}$ . Total group number,

according to the classification of the Society of American Bacteriologists—211.2442533.

CO rods,  $2-4\mu \times 1\mu$ , occurring singly, motile, with peritrichous flagella, do not form spores, gram negative; agar streak—growth good, smooth, opalescent, medium unchanged; colonies on Thornton's medium—round flat, bluish translucent, edges undulate; liquefies gelatine quickly, produces acid on dextrose, saccharose, and glycerine, has no diastatic action on starch, reduces nitrates to nitrites; optimum temperature about  $25^{\circ}\text{C}$ . Total group number—211.2422032.

As a control, a mixed culture of the strains YB and CO was made from a soil extract culture in which the *Colpidia* had died. It was enriched with YB at each sub-culturing, in the same way as the *Colpidium* cultures, and a sub-culture of the same age as the corresponding *Colpidium* culture was used for each inoculation.

The method of inoculation employed was the same in every case; the numbers of bacteria in both parent cultures were estimated by a direct count. Volumes of each parent culture containing approximately the same bacterial numbers were then removed with a sterile pipette and added to the experimental flasks; in most cases 1 c.c. of the control culture, and 3 or 4 c.c. of the *Colpidium* culture, were taken. In the case of the control culture sterile soil extract was afterwards added to bring the final volume of liquid to the same level in both flasks.

*Methods of counting.* The counts in all cases were by the direct method. The bacteria in duplicate loopfuls were counted with a Thoma haemocytometer, 1 organism per small square representing a bacterial content of 20 million per c.c.: while the counting chamber used for estimating the numbers of *Colpidia* was of the Cropper type, 0.1 mm. in depth, with a ruled area of 25 sq. mm. divided into 625 small squares; owing to the small numbers of *Colpidia* it was usually necessary to make five counts on each occasion.

## PART I. PEPTONE EXPERIMENTS.

### *Methods.*

The medium used was a 0.5 per cent. solution of peptone (Bacteriological, B.D.H.), in soil extract. The soil extract was made by adding 2 litres of water to 1 kg. of soil, boiling for  $1\frac{1}{2}$  hours and filtering. In most cases the required volume of freshly made soil extract was placed in a conical flask and sterilised once in the autoclave, the peptone added, and the whole then steamed for 1 hour. In two cases (experiments 6 and

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7), the soil extract used had been sterilised three times in 10 c.c. portions, before the peptone was added.

Eight experiments in all were performed, in the first three 60 c.c. of medium for each culture were used in a 250 c.c. conical flask, in the fourth and fifth 30 c.c. of medium in 100 c.c. flasks, and in the last three 50 c.c. in 250 c.c. flasks. In all cases the flasks were plugged with cotton wool and kept on the bench at room temperature. The full period of these experiments was a fortnight, but experiments 4, 5 and 8 were only continued for a week. Counts of bacteria and protozoa were made daily by the method described above.

The ammonia present in the cultures was determined at weekly intervals, on duplicate samples of 1 c.c. by the method of Woolf(9); the strength of the acid used for the titration was about 0.005 N, 1 c.c. being equivalent to 0.0711 mgm. of nitrogen as ammonia.

In one case (experiment 4) an additional ammonia estimation was made on the third day after inoculation.

### *Results.*

As an illustration of the kind of results obtained, the course of two experiments (1 and 7) is shown in diagram form. In experiment 1 (Fig. 1)

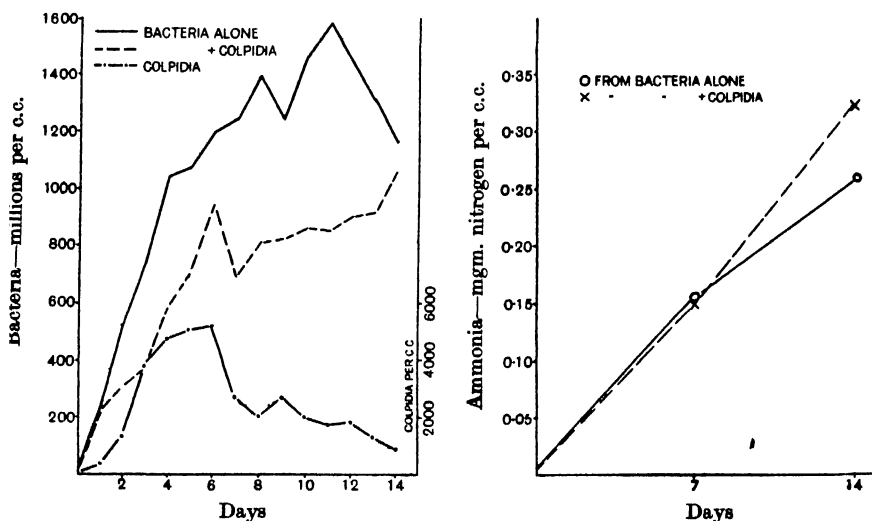


Fig. 1. Numbers of organisms and ammonia produced in experiment 1.

the Colpidia grew very well, reaching a maximum number of 5200 per c.c. and being still alive in appreciable numbers at the end of the second week

of the experiment. In consequence of this good growth of *Colpidia*, it will be seen that the numbers of bacteria were much reduced in the *Colpidium* culture; during practically the whole course of the experiment they remained below 1000 million per c.c., whereas in the control culture the numbers rose to 1580 million per c.c. and were above 1000 million from the fourth day onwards.

The ammonia present in the two cultures was practically the same in amount at the end of one week, and at the end of the second week more ammonia was present in the *Colpidium* culture than in the control.

Experiment 7, on the other hand, is an example of an experiment where the *Colpidia* did not grow well (Fig. 2). In this case they reached a

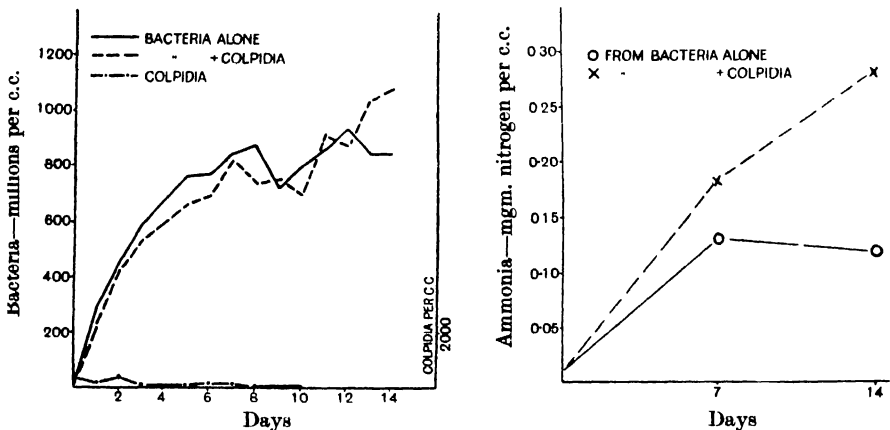


Fig. 2. Numbers of organisms and ammonia produced in experiment 7.

maximum of 320 per c.c. and at the end of a week were dying off, being reduced to 160 per c.c., and were all dead by the tenth day. The numbers of bacteria accordingly followed very nearly the same course in both cultures. There was, however, a greater amount of ammonia produced in the first week in the *Colpidium* culture than in the control; and this increase is seen to be continued in the second week, though, as some ammonia was lost from the control culture in the second week, the final estimation in this culture does not represent the full amount of ammonia formed.

The increase in ammonia in both sets of cultures during the first week of each experiment, and the average of the bacterial numbers over the first week, are shown in Table I (experiment 5 is omitted, for reasons to be explained).

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Table I.

Experiment	Control cultures		<i>Colpidium</i> cultures	
	Average no. of bacteria (millions per c.c.)	Increase in ammonia (mgm. nitrogen per c.c.)	Average no. of bacteria (millions per c.c.)	Increase in ammonia (mgm. nitrogen per c.c.)
1	755	0.14	453	0.14
2	397	0.07	507	0.14
3	386	0.06	352	0.14
4	409	0.16	312	0.15
6	540	0.14	445	0.15
7	532	0.12	480	0.17
8	597	0.13	562	0.17
Average of all figures	517	0.12	444	0.15

It will be seen from Table I, that, though the *Colpidia* had reduced the numbers of bacteria as compared with those in the control cultures, yet the cultures containing *Colpidia* produced slightly more ammonia during the first week.

Since previous work on carbon dioxide production by Cutler and Crump(3), and on ammonia production by the writer(6), has shown that the efficiency of the individual organisms is a function of the numbers of bacteria present, it was decided to examine the relation between the numbers of bacteria in any culture and the ammonia produced in that culture. The method formerly used(6) was again employed; the efficiency of the individual organism was compared against the number of organisms, the amount of ammoniacal nitrogen produced in 24 hours per 1000 million bacteria being used as a measure of efficiency.

Table II.

Experiment	Control cultures			<i>Colpidium</i> cultures		
	<i>n</i>	<i>P</i>	<i>Q</i>	<i>n</i>	<i>P</i>	<i>Q</i>
	Average no. of bacteria (millions per c.c.)	Ammonia per 24 hours (mgm. nitrogen per c.c.)		Average no. of bacteria (millions per c.c.)	Ammonia per 24 hours (mgm. nitrogen per c.c.)	
1. 1st week	755	0.0202	0.0268	453	0.0197	0.0434
2nd "	1353	0.0148	0.0110	864	0.0242	0.0280
2. 1st "	397	0.0093	0.0235	507	0.0193	0.0397
2nd "	905	0.0207	0.0228	931	0.0205	0.0220
3. 1st "	386	0.0083	0.0216	352	0.0195	0.0554
2nd "	868	0.0171*	0.0197*	733	0.0163	0.0222
4. 1st "	409	0.0229	0.0560	161†	0.0220	0.1367
				467‡	0.0212	0.0454
5. 1st "	498	0.0210*	0.0421*	454	0.0276	0.0608
6. 1st "	540	0.0198	0.0367	445	0.0214	0.0481
7. 1st "	532	0.0170	0.0320	480	0.0245	0.0511
8. 1st "	597	0.0190	0.0318	562	0.0240	0.0428

\* Values omitted from calculation.

† First 3 days.

‡ 4th-7th days.

In Table II, the values of average bacterial numbers ( $n$ ), ammonia produced per 24 hours ( $P$ ), and efficiency ( $Q$ ), are given for both sets of cultures.

From an inspection of Table II it will be seen, firstly, that the values of  $Q$  are higher, as a rule, for the *Colpidium* cultures, with an average value for  $Q$  of 0.0495, as against an average value of 0.0280 from the control cultures.

The second point to be noticed is that, on the whole, higher values of  $Q$  correspond to lower bacterial numbers in both sets of cultures. From this it appears that there probably is an inverse mathematical relation between  $Q$  and  $n$  (average bacterial numbers), and as the simplest case it may be presumed that this relation is a linear one. To test whether this relation does in fact exist, and is in fact linear, the regression of  $Q$  upon  $n$  was calculated.

On taking the figures for both sets of cultures together (except those marked \* in Table II), the following results were obtained:

Mean value of  $Q = 0.0408$ , if  $n = 585$ .

Regression coefficient of ( $Q \times 10^4$ ) on  $n = -0.6488$ .

Estimated standard error of regression coefficient = 0.1711.

From this value for the standard error, by applying Fisher's  $t$  test (4) the regression coefficient is found to be significantly different from zero, thus showing that the inverse relation presumed to exist between  $Q$  and  $n$  is a real one, and is linear. This relation can be expressed by the equation

$$Q \times 10^4 = 788 - 0.649 n.$$

On account of the small number of cases (only 21 for both series taken together) it was not possible, by investigating each series separately, to find whether the relation between  $Q$  and  $n$  in this case was altered by the presence of *Colpidia*.

It will have been noticed that two sets of figures in the control series (marked \*) have been omitted from the calculation. This was on account of the detection of appreciable amounts of nitrite in these cultures; the causes of its formation are obscure, but as it was probably formed by the oxidation of ammonia, it is clear that the ammonia present in these cultures did not represent the total amount produced.

*Numbers of Colpidia.* It will be clear, from what has been said above, that the *Colpidia* did not grow equally well in all cases. While some of these differences in growth may be attributed to differences in the medium, their principal cause is undoubtedly the size and age of the inoculum, as is shown in Table III. In this table the cultures are arranged in order of



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the maximum numbers of Colpidia that were observed to be present in them.

Table III.

Experiment	Maximum no. per c.c. of Colpidia observed	Total no. of Colpidia in inoculum	Age of parent culture (days)	Life of Colpidia in experiment (days)
1	5200	22,000	4	14 +
8	1760	18,000	4	7 +
3	960	11,000	4	14 +
6	960	21,000	7	14 +
7	320	18,000	7	10
2	160	6,000	4	10
4	80	2,500	5	7
5	25	480	5	6

(Where a + sign is added in the last column, it means that the Colpidia were still alive on this day, which was the last on which observations were made.)

It will be seen that the maximum numbers attained follow the same order as the total number in the inoculum, except in the case of cultures 6 and 7, where the parent culture was older, and the maximum numbers attained were accordingly not so great as those from a younger parent culture of the same density. This dependence of the numbers of Colpidia attained on the size and age of the inoculum was recorded by Cutler and Crump (2).

### *Summary of results.*

The results obtained from this first series of experiments may be summarised as follows:

1. In the presence of Colpidia the bacterial numbers are depressed, but a slightly greater amount of ammonia is produced.
2. Taking both sets of cultures together, an inverse linear relation is found to hold between average bacterial numbers and the amount of ammonia produced per organism.
3. The numbers of Colpidia in the experimental cultures were chiefly determined by the size of the inoculum and the age of the parent culture.

## PART II. ALANINE EXPERIMENTS.

### *Methods.*

The medium used was a 0.2 per cent. solution of alanine ( $\alpha$  amino-propionic acid) in a mineral salt solution of the following composition: NaCl 0.06 per cent., KCl 0.001 per cent.,  $\text{CaCl}_2$  0.002 per cent.,  $\text{MgSO}_4$  0.001 per cent.,  $\text{KH}_2\text{PO}_4$  0.12 per cent., made up in ammonia-free distilled water. 50 c.c. portions of this medium were placed in 250 c.c. conical flasks, and steamed for 1 hour; the pH of the medium was then adjusted

to 7.2 by the addition of  $N/10$  sodium hydroxide, and it was steamed again for 1 hour.

Ten experiments in all were performed; the full period of an experiment was 2 weeks, but several experiments were not continued beyond the first week. The flasks were kept at room temperature, and in the first two experiments were plugged with cotton wool, but not otherwise aerated. In all the other experiments a slow stream of carbon dioxide-free air, filtered through cotton wool, was drawn through the liquid by an aspirator, at a rate of 5 to 7 litres of air per 24 hours. The carbon dioxide in the outgoing air was determined by the Pettenkofer method; a baryta solution of about 0.25 per cent. strength was used, and was titrated with approximately  $N/10$  hydrochloric acid of known strength. Titrations were made at least once every 24 hours, and more often where necessary.

The amount of ammonia present in each culture was determined, by Woolf's method, on the third or fourth day of each experiment, at the end of a week, and at the end of 2 weeks when required. To detect any ammonia given off from the cultures, the outgoing air from each flask was passed through a trap containing 3 per cent. boric acid and 0.002 per cent. brom-cresol green.

### *Results.*

In the first two experiments, in which the flasks were plugged with cotton wool, considerable amounts of nitrite were found in the control cultures, and the results of these experiments were accordingly rejected.

The results considered here are, then, those of the last eight experiments, of which three (experiments 3, 5 and 6) were continued for a fortnight, the others for a week only.

*Growth of organisms.* The course of events in a single experiment is illustrated in the following diagram of experiment 6 (Fig. 3). This experiment shows a double maximum in bacterial numbers in the control cultures, which was not observed in any other case, but is otherwise typical.

It will be seen that the *Colpidia* reach a maximum number of a little over 2000 per c.c. and survive till the eighth day. During the period when the *Colpidia* were active—the first 6 days—the numbers of bacteria were less in the *Colpidium* culture than in the control. In both cultures the numbers of bacteria rose till the tenth day, and fell off markedly after this point.

These two phenomena, the reduction of bacterial numbers by *Colpidia* in the first week, and the falling off in numbers during the second week, occurred in every case, and were borne out by the appearance of the cultures themselves.

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The control cultures became progressively more turbid during the first few days, and a yellow colour developed in the medium from the fifth or sixth day onwards. In the *Colpidium* cultures, although an increasing turbidity was visible, it was not so great as that in the control cultures, and from the third to the fifth or sixth day the difference in turbidity between the two cultures was well marked; the yellow colour too began to develop a day or two later in the *Colpidium* cultures than in the controls. Towards the middle of the second week all the cultures

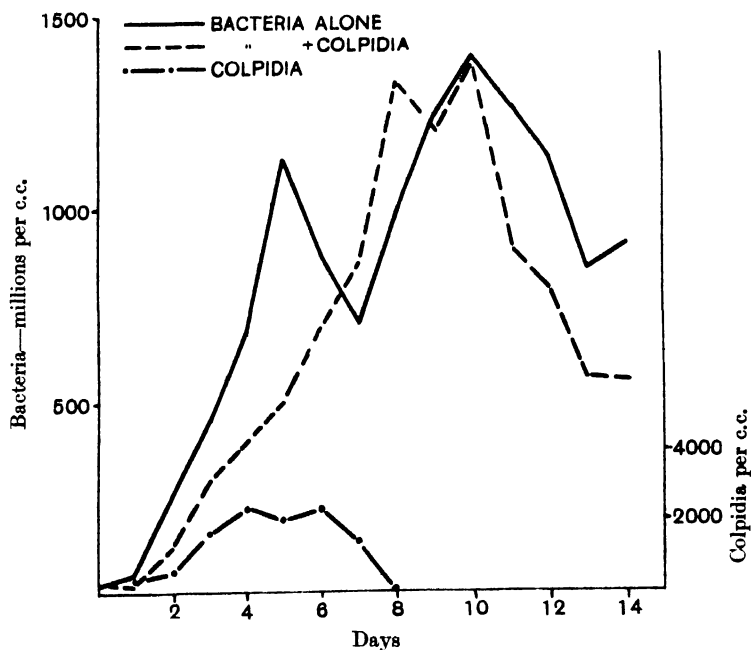


Fig. 3. Numbers of organisms in experiment 6.

began to clear, and the typical appearance of an old culture kept beyond the period of the experiment was of a clear, deep yellow, supernatant fluid, with a much reduced flocculent mass of bacteria on the bottom of the flask.

The normal period during which *Colpidia* survive in the cultures seems to be 7 or 8 days, though in the last three experiments there were still over 1000 *Colpidia* per c.c. present at the end of a week, when the experiments were stopped. The maximum number of *Colpidia* recorded in individual cultures ranged from 2240 to 6400 per c.c. with an average of 1400 per c.c.

Though the reduction in bacterial numbers by the *Colpidia* was very well marked, in most cases amounting to 40 to 60 per cent. of the control numbers during the first few days, yet there did not seem to be any simple relation between the amount of this reduction and the numbers of *Colpidia* present. This was probably due to the fact that the size of the *Colpidia* decreased with increasing age and increased crowding in the cultures.

Though the *Colpidia* reduced the total numbers of bacteria, they did not seem to affect the proportion of the two kinds of bacteria present. In the direct counts, the proportion of YB to CO bacteria in both series of cultures seemed to be about 3 to 1, and this was borne out by the relative numbers obtained in the plate count carried out on experiment 10.

On the fifth, sixth and seventh days of this experiment, in the control plates the CO colonies were 21 per cent., 29 per cent. and 26 per cent. of the total colonies, and in the *Colpidium* plates 28 per cent., 30 per cent. and 19 per cent. of the total.

*The evolution of carbon dioxide.* The amounts of carbon dioxide evolved from day to day in experiment 6 are shown in Fig. 4. In the control culture the carbon dioxide increased in amount till a maximum was reached on the fourth day of the experiment; the peak in the initial rise of bacterial numbers was observed on the fifth day. The amount of carbon dioxide then fell, to rise to a second peak on the eighth day, 2 days before the second peak in the bacterial numbers, observed on the tenth day. This occurrence of the maximum production of carbon dioxide a day before the maximum numbers of bacteria has been recorded by Cutler and Crump<sup>(3)</sup> and Telegdy-Kovats<sup>(8)</sup> in sand cultures, and was observed in eight cases in the series of experiments under discussion, five of the eight being control cultures.

In this connection it may be noted that an attempt was made to find whether any simple mathematical relation existed between the growth rate of the bacteria in a culture over a particular day, and the amount of carbon dioxide produced on that day. This attempt was not successful; in this series of cultures there appears to be no direct relation between the growth rate of the bacteria and the carbon dioxide produced by them.

The *Colpidium* culture evolved a greater amount of carbon dioxide than the control during the first 3 days, and the amount given off increased till the fourth day, when a slight fall was followed by a rise to a maximum value on the seventh day, corresponding to a peak in bacterial numbers on the eighth day. In both cultures the amount of carbon dioxide evolved fell off very much in the second week.

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*Efficiency relations.* In studying the efficiency of the individual organism in the production of carbon dioxide, the amount of carbon dioxide produced per 24 hours per 1000 million bacteria ( $Q$ ) was taken as a measure of efficiency. In Fig. 5 are shown the changes in this quantity, and in the average numbers of bacteria over every 24 hours, during the course of growth of a single culture, the control culture in experiment 5.

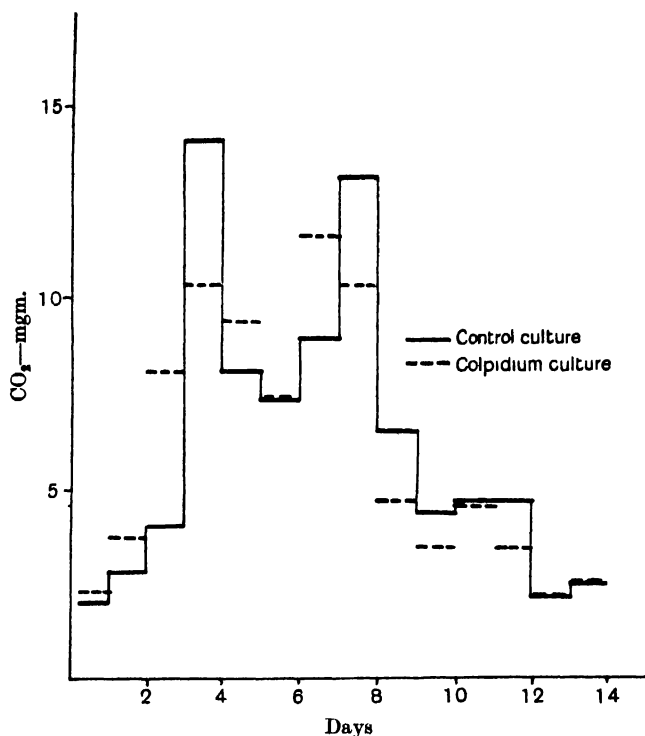


Fig. 4. Rate of evolution of CO<sub>2</sub>—experiment 6.

The average bacterial numbers increased till the eighth day and then decreased; the efficiency fell while the bacterial numbers were increasing, and thereafter remained at a low and approximately constant level.

It is evident that the relation between efficiency and bacterial numbers undergoes a change after the bacterial numbers have reached their highest point and begun to decrease. It was therefore decided to study in detail only the period in which the bacterial numbers are still increasing, more particularly as the *Colpidia* were alive in the cultures during the earlier period only. The values of average bacterial numbers ( $n$ ) carbon dioxide produced per c.c. per 24 hours ( $P$ ) and efficiency ( $Q$ ), up to the

highest point of increase of the bacterial numbers, are given for six cultures (3-8), in Table IV. The quantity ( $Q$ ) used as a measure of efficiency was calculated in each case from the equation

$$Q = \frac{P \times 1000}{n}.$$

The figures for the first 24 hours are omitted from the calculation in every case for the *Colpidium* cultures, and in two cases for the control cultures (omitted figures marked \*), on account of the high possibility of error in the carbon dioxide estimation in these cases.

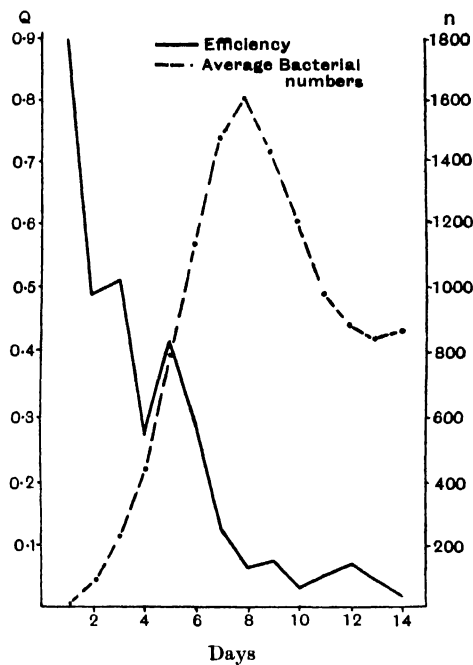


Fig. 5. Experiment 5—control culture.

The data were examined statistically with the following results:

*Control cultures.* Average value of  $Q = 0.3219$ , if  $n = 830$ .

Regression coefficient of  $(Q \times 10^3)$  on  $n = -0.3065$ .

Estimated standard error of regression coefficient = 0.05515.

Number of cases = 41.

From this value for the standard error, the regression coefficient is found by the  $t$  test to be significantly different from zero, and the relation

Table IV.

*Carbon dioxide.*

Exp.	Days	Control cultures			Colpidia cultures		
		<i>P</i> CO <sub>2</sub> per c.c. per 24 hours	<i>n</i> Average bacterial numbers (millions)	<i>Q</i>	<i>P</i> CO <sub>2</sub> per c.c. per 24 hours	<i>n</i> Average bacterial numbers (millions)	<i>Q</i>
3	0-1	0.0835	168.5	0.4955	0.0877	50	1.7540*
	1-2	0.1183	568	0.2083	0.1023	249	0.4108
	2-3	0.1981	1194	0.1659	0.2749	456	0.6029
	3-4	0.5850	1660	0.3524	0.2590	492	0.5264
	4-5	0.1279	1790	0.0715	0.2461	792	0.3107
	5-6	0.0831	1830	0.0454	0.1375	1282	0.1073
	6-7	0.0735	1835	0.0401	0.1693	1960	0.0864
	7-8				0.0799	2280	0.0350
4	0-1	0.0376	91	0.4132	0.0541	26	2.0810*
	1-2	0.1169	265	0.4411	0.0734	73.5	0.9986
	2-3	0.3421	680	0.5031	0.1087	268	0.4056
	3-4	0.4427	1430	0.3096	0.3012	624	0.4827
	4-5	0.0869	2060	0.0422	0.1773	940	0.1886
	5-6				0.2018	1330	0.1517
	6-7				0.1278	1750	0.0730
5	0-1	0.0162	18	0.9000	0.0362	15	2.4130*
	1-2	0.0439	90	0.4878	0.0596	28	2.1290
	2-3	0.1198	234	0.5120	0.0892	94	0.9489
	3-4	0.1183	436	0.2713	0.1822	229	0.7956
	4-5	0.3268	788	0.4147	0.3127	438	0.7139
	5-6	0.3302	1148	0.2876	0.2487	794	0.3132
	6-7	0.1881	1480	0.1271	0.1881	1235	0.1523
	7-8	0.0998	1610	0.0620	0.1238	1595	0.0776
6	0-1	0.0501	33	1.5180	0.0580	20	2.9010*
	1-2	0.0539	150	0.3593	0.0719	72	0.9986
	2-3	0.0770	354	0.2175	0.1540	208	0.7404
	3-4	0.2665	566	0.4708	0.1954	342	0.5713
	4-5	0.1616	900	0.1796	0.1864	448	0.4161
	5-6	0.1461	1000	0.1461	0.1461	600	0.2435
	6-7	0.1771	796	0.2225	0.2299	780	0.2947
	7-8	0.2888	862	0.3350	0.2269	1098	0.2066
	8-9	0.1443	1126	0.1282	0.1032	1266	0.0815
	9-10	0.0952	1312	0.0726	0.0748	1296	0.0577
	10-11	0.1021	1324	0.0771			
7	0-1	0.0443	23	1.9250*	0.0262	12	2.1800*
	1-2	0.0652	65	1.0030	0.0591	28	2.1090
	2-3	0.0632	212	0.2981	0.0632	63	1.0030
	3-4	0.1643	386	0.4258	0.1598	179	0.8926
	4-5		604			270	
	5-6	0.3420	792	0.4318	0.2611	358	0.7293
	6-7	0.2115	932	0.2270	0.2026	556	0.3644
	7-8	0.2337	1172	0.1994	0.2737	724	0.3781
8	0-1	0.0768	22	3.4900*	0.0536	16	3.3490*
	1-2	0.0939	72	1.3040	0.0819	38	2.1560
	2-3	0.1280	186	0.6878	0.0960	76	1.2640
	3-4	0.1280	346	0.3697	0.1559	130	1.2000
	4-5	0.1351	520	0.2597	0.1371	230	0.5959
	5-6	0.1475	704	0.2095	0.2057	384	0.5356
	6-7	0.2035	966	0.2106	0.2472	604	0.4093

\* Values omitted from calculations.

between  $Q$  and  $n$  for the control cultures can be expressed by the equation

$$Q \times 10^4 = 5763 - 3.065n.$$

*Colpidium* cultures. Average value of  $Q = 0.6039$ , if  $n = 644$ .

Regression coefficient of  $(Q \times 10^3)$  on  $n = -0.6819$ .

Estimated standard error of regression coefficient =  $0.1045$ .

Number of cases =  $41$ .

In this case also the regression coefficient is significantly different from zero, and the relation between  $Q$  and  $n$  for the *Colpidium* cultures can be expressed by the equation

$$Q \times 10^4 = 10430 - 6.819n.$$

The relation between efficiency and bacterial numbers in the case of carbon dioxide production is therefore of the same kind as that formerly recorded for ammonia production, that is, that  $Q$  falls off regularly for increasing values of  $n$ .

Though the two sets of cultures show the same kind of relation between  $Q$  and  $n$ , yet there is a significant difference between the regression coefficients for the control and the *Colpidium* cultures, as is shown in Table V.

Table V.

	sion coefficient	Estimated standard error	$t$	Degrees of freedom
Control cultures	- 0.3065	0.0552	5.56	39
<i>Colpidium</i> cultures	- 0.6819	0.1045	6.53	39
Difference	0.3754	0.1181	3.18	—

The value for  $t$  obtained for the difference shows it to be significant.

In Fig. 6 the calculated regression lines for both sets of cultures are shown, with the observed values represented by dots. In the case of the control cultures, the dots are evenly distributed about the line, which can be considered to represent adequately the relation between  $Q$  and  $n$ . With the *Colpidium* cultures, however, the points do not fit so well, particularly at very low or very high values of  $n$ , and although it was assumed, for purposes of calculating the regression, that the relation between  $Q$  and  $n$  was a linear one, it is probable that this relation would be more accurately expressed by a hyperbolic curve.

*Effect of time.* It will be noticed in Fig. 5 that the average bacterial numbers are increasing with time over the period studied, while the efficiency is decreasing. It may therefore be doubted whether the negative relation found to hold between  $Q$  and  $n$  may not be entirely due to the effect of time on these two quantities.



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To test this point the observations for each set of cultures were grouped in days, and the variations from one day to another separated from the variations within days, when the following results were obtained (Table VI).

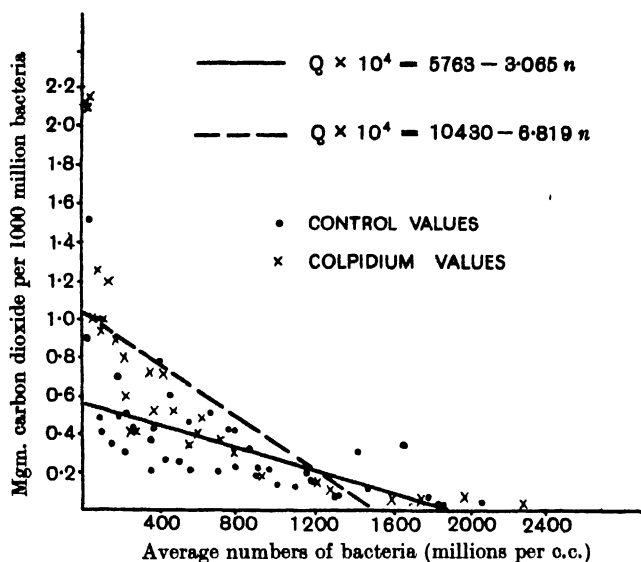


Fig. 6. Carbon dioxide—relation between bacterial numbers and efficiency.

Table VI.

	Number of days	Regression coefficient	Estimated standard error	<i>t</i>	Degrees of freedom
Control cultures	11				
Total		-0.3065	0.0552	5.56	39
Within days		-0.1864	0.0765	2.44	29
Colpidium cultures	9				
Total		-0.6819	0.1045	6.53	39
Within days		-0.4270	0.1566	2.73	31

By applying the *t* test(4) the regression coefficients due to variation within days are seen to be significant.

When the effect of time is eliminated there remains in both cases a significant negative relation between *Q* and *n*; that is to say, that though a culture 7 days old will have a lower efficiency than one 4 days old, yet of two 4-day-old cultures the one with the lower bacterial numbers will have the higher efficiency.

There are not sufficient data to show whether the regression independent of time is significantly different in the two sets of cultures.

*Ammonia production.* The results obtained for ammonia production from this series of cultures were very similar to those obtained on peptone. The eight control cultures produced an average of 0.19 mgm. of ammonia nitrogen per c.c. in one week, with an average bacterial content for all control cultures of 662 millions per c.c. In the *Colpidium* cultures the average ammonia nitrogen produced at the end of one week was 0.18 mgm. per c.c. from an average bacterial content of 398 millions per c.c. for all cultures.

In the *Colpidium* cultures, therefore, although the bacterial numbers were sensibly reduced, the amount of ammonia produced was nearly the same as from the control cultures.

The values of average bacterial numbers ( $n$ ), ammonia nitrogen per c.c. produced per 24 hours ( $P$ ) and efficiency ( $Q$ ) for both series of cultures are given in Table VII.

The quantity  $Q$  used as a measure of efficiency was calculated as before. (Ammonia nitrogen per 24 hours per 1000 million bacteria.)

Table VII.

*Ammonia nitrogen.*

Control cultures					<i>Colpidium</i> cultures		
Exp.	Days	$n$	$P$	$Q$	$n$	$P$	$Q$
		Average bacterial numbers (millions)	Ammonia $N$ (mgm. per c.c.)		Average bacterial numbers (millions)	Ammonia $N$ (mgm. per c.c.)	
3	1-7	1247	0.0343	0.0275	815	0.0314	0.0386
4	1-3	386	0.0571	0.1479	147	0.0255	0.1735
	3-7	1702	0.0135	0.0079	1162	0.0381	0.0328
5	1-3	126	0.0193	0.1532	54	0.0135	0.2500
	3-4	436	0.0324	0.0743	229	0.0298	0.1301
	4-7	1134	0.0445	0.0392	837	0.0545	0.0651
6	1-4	291	0.0230	0.0790	169	0.0199	0.1177
	4-7	848	0.0269	0.0317	614	0.0351	0.0572
7	1-3	118	0.0102	0.0864	37	0.0054	0.1459
	3-7	681	0.0382	0.0561	384	0.0335	0.0963
8	1-4	169	0.0178	0.1053	69	0.0117	0.1688
	4-7	743	0.0310	0.0418	417	0.0366	0.0877
9	1-7	500	0.0218	0.0435	171	0.0161	0.0939
10	1-7	387	0.0198	0.0512	186	0.0177	0.0953

A statistical analysis of the two sets of data gave the following results:

*Control cultures.* Average value of  $Q = 0.0675$ , if  $n = 627$  million.

Regression coefficient of ( $Q \times 10^4$ ) on  $n = -0.707$ .

Estimated standard error of regression coefficient = 0.1745.

$t = 4.05$ .

Number of cases = 14.

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*Colpidium* cultures. Average value of  $Q = 0.1109$ , if  $n = 376$  millions.

Regression coefficient of  $(Q \times 10^4)$  on  $n = -1.35$ .

Estimated standard error of regression coefficient = 0.2976.

$t = 4.54$ .

Number of cases = 14.

Difference in regression coefficients = 0.643.

Estimated standard error of difference = 0.2411.

$t = 2.667$ .

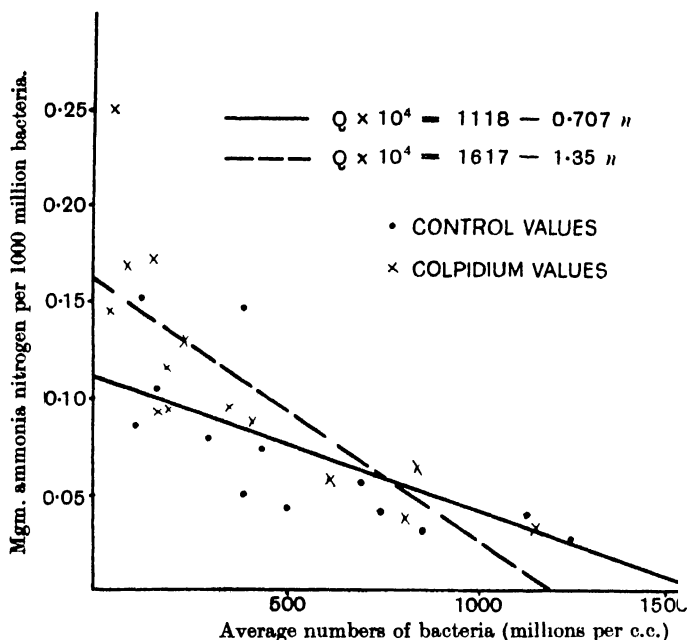


Fig. 7. Ammonia—relation between bacterial numbers and efficiency.

From the values of  $t$  in each case it is apparent that there exists a significant negative relation between  $Q$  and  $n$  for both the *Colpidium* and the control cultures, and that the two regressions are significantly different.

The relation between  $Q$  and  $n$  for the control cultures can be expressed by the equation

$$Q \times 10^4 = 1118 - 0.707n,$$

and for the *Colpidium* cultures by the equation

$$Q \times 10^4 = 1617 - 1.35n.$$

In Fig. 7 the actual observations for both sets of cultures are shown together with the calculated regression lines, which seem to give a fairly accurate picture of the true relation between  $Q$  and  $n$ .

There are not sufficient data to discover how much of the variations of  $Q$  and  $n$  are due to the effect of time, and to eliminate this effect.

*Changes in medium during course of experiment.* In two experiments (9 and 10), in which  $pH$  measurements were made daily by the capillator method, the cultures were found to become steadily more alkaline, the  $pH$  changing from 7.1 at the start to about 8.0 at the end of a week. The alkalinity corresponds roughly to the amount of ammonia present, the culture that contains more ammonia being more alkaline.

In Fig. 8 are shown the total carbon dioxide and total ammonia nitrogen produced up to any day in experiment 6. The scale on which each product is plotted has been adjusted so that two mols of carbon dioxide correspond to each mol of ammonia. It is evident that at the end of a fortnight the reactions taking place in the culture are practically completed; the bacteria have begun to die off before this, and the carbon dioxide produced per day has fallen to a very low value.

If all the carbon in the medium were converted into carbon dioxide the total carbon dioxide evolved would be about 148 mgm.; the amount of carbon dioxide actually evolved in a fortnight corresponds to two-thirds of this figure. In experiment 6, for instance, two-thirds of the total carbon as carbon dioxide would give 89.8 mgm. of carbon dioxide in all (allowing for carbon lost in sampling). The total carbon dioxide actually evolved in a fortnight was 85.6 mgm. from the control culture, and 84.2 mgm. from the *Colpidium* culture. At the completion of the reaction, or set of reactions, carried out by the bacteria, two-thirds of the total carbon is accounted for as carbon dioxide. As will be seen from Fig. 8, during the first week one mol of ammonia is produced for every two mols of carbon dioxide, as the points representing ammonia produced lie along the curve of carbon dioxide production. Now the alanine molecule contains three carbon atoms to one nitrogen atom—its structural formula being  $CH_3CHNH_2COOH$ , and the proportion of carbon dioxide to ammonia produced suggests that the main reaction taking place is one that involves the break up of the carbon chain, two carbon atoms being lost as carbon dioxide.

With regard to the ammonia, it should be emphasised that the ammonia recorded was all present in the medium; in no case was there any loss of ammonia from the cultures during the first week, and only very small losses were recorded by the acid traps in the second week. It is possible that the ammonia is present in the medium as ammonium bicarbonate, and that the third carbon atom from each alanine molecule is also accounted for in the formation of this compound. This supposition

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is rendered more probable by the increasing alkalinity of the medium, and also by the fact that the filtrate from an old culture, on titration to pH 6.0 and subsequent aeration in the cold, gave off an appreciable

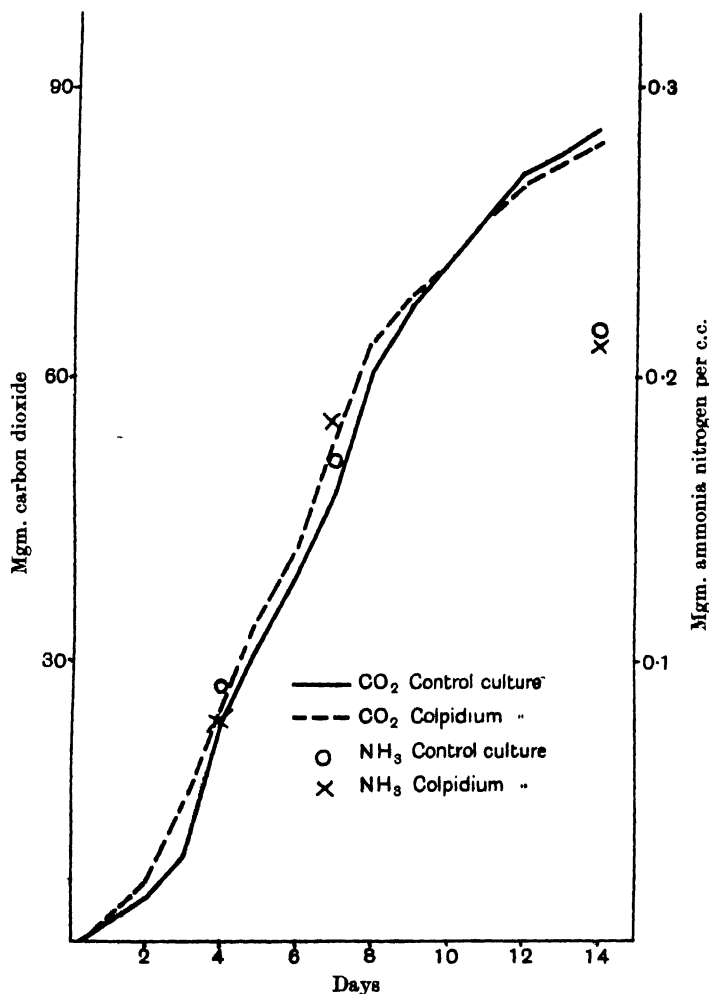


Fig. 8. Total carbon dioxide and ammonia in experiment 6.

amount of carbon dioxide; a solution of ammonium carbonate of corresponding strength behaved in the same way.

That there were other reactions taking place in the culture besides this complete breakdown with formation of ammonium bicarbonate, is shown by the fact that in the residue from an old culture there are

present a volatile reducing substance, probably acetone, and small amounts of volatile acids, possibly acetic and formic acids, as salts.

Of the carbon not accounted for as carbon dioxide, only a very small proportion is used in the formation of bacterial protoplasm. If it is assumed that 20 per cent. of the total volume of the bacteria is solid matter, and that 50 per cent. of this is carbon, then at the greatest concentration of bacteria in these experiments, 2000 million per c.c., the weight of carbon in the substance of the bacteria is only 0.2 mgm., whereas there are 13.5 mgm. of carbon in the medium not accounted for as carbon dioxide.

It is of interest to note that the ratio  $\frac{\text{nitrogen as ammonia}}{\text{carbon as carbon dioxide}}$  is higher at the end of a week in the control culture than in the *Colpidium* culture in every case but one; the mean value of this ratio is 0.685 for the control cultures, and 0.596 for the *Colpidium* cultures, and the difference, though small, is significant.

#### DISCUSSION.

On considering the foregoing results, there are two main conclusions which emerge. The first is that in any one series of experiments, the amount of ammonia, or of carbon dioxide, produced per individual organism (efficiency) is found to fall off regularly as the bacterial numbers increase. The regression of efficiency on bacterial numbers is represented very nearly by a straight line, except in the case of carbon dioxide production in *Colpidium* cultures, where the values of efficiency for very low bacterial numbers are higher than would be expected, and a curved regression line would fit the data better. This raising of efficiency for low bacterial numbers may be partly due to the respiration of the *Colpidia*, but it does not show any relation to the numbers of *Colpidia* present.

The inverse relation between efficiency and bacterial numbers agrees with the results recorded in a previous paper<sup>(6)</sup>, although the present experiments were not performed with a pure culture of bacteria, and were of much shorter duration than in the former case.

The second conclusion is that, although the numbers of bacteria in the *Colpidium* cultures are markedly lower than in the controls, yet the two sets of cultures produce nearly equal amounts of ammonia or of carbon dioxide; that is, that the *Colpidium* cultures show a higher efficiency. Now it might be supposed that, since efficiency is inversely proportional to bacterial numbers, the higher efficiency observed in the

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*Colpidium* cultures was due entirely to their lower bacterial numbers. If this were so, then one would expect the same regression line of efficiency on bacterial numbers to fit the data from both *Colpidium* and control cultures. The two sets of cultures, however, give regression lines which are significantly different; the effect of the presence of *Colpidia* on ammonia or carbon dioxide production is not entirely due to the reduction of bacterial numbers to a point nearer the optimum level for metabolic activity. There remain two explanations for this increased efficiency; the extra carbon dioxide or ammonia may not be produced by the bacteria, but by the *Colpidia*; or the presence of the *Colpidia* may exert a stimulating effect on bacterial activity, over and above that caused by the lower numbers of bacteria present. The first alternative involves the assumption that the *Colpidia* are partially saprophytic in their method of feeding; the quantities of carbon dioxide and ammonia produced are too large to be the products of respiration and excretion of a holozoic organism. The majority of work on the feeding habits of *Colpidium*, however, seems to show that it is undoubtedly holozoic; Cutler and Crump<sup>(2)</sup> found that the rate of growth of a *Colpidium* culture was dependent on the number of bacteria supplied to it, and Oehler<sup>(7)</sup>, who made several attempts to grow it in bacteria-free media, though he obtained sterile specimens, never succeeded in getting growth and multiplication except with living bacteria as a source of food. Luck, Sheets and Thomas<sup>(5)</sup>, however, report that they have cultivated *Colpidium* in bacteria-free media, but give no details. In the present series of cultures, the marked reduction in bacterial numbers produced by the *Colpidia*, and the presence of bacteria in their food vacuoles, suggest that the *Colpidia* are predominantly, if not entirely, holozoic.

There remains the possibility of a stimulating effect due to the presence of *Colpidia*. Such a stimulus might be due to the formation by the protozoa of an excretion product (e.g. urea), which was more easily utilised by the bacteria than the original substrate. The existence of such an excretion product, though quite possible, cannot be detected, as it would be decomposed by the bacteria as fast as it was formed. Apart from the possibility of such an excretion product, the *Colpidia* may exert a stimulating action by keeping the cultures in which they are present in a state of physiological youth over a longer period than the normal. A normal culture of bacteria shows a high level of metabolic activity during the early stages, in which the bacteria are actively dividing, but in the later stages, when the multiplication of bacteria slows down, and eventually stops, this activity is considerably lessened. If protozoa are present,

however, they exert what may be described as a "pruning" action on the culture; by removing bacteria from the culture by feeding, they are compelling the remaining bacteria to divide more rapidly. A culture in which protozoa were present would therefore be in a state of active division over a longer period than the normal, and might therefore exhibit the high metabolic activity of a very young culture during this period. This hypothesis was put forward by Cutler and Bal<sup>(1)</sup> to explain the increase in nitrogen fixation by *Azotobacter* due to the presence of *Colpidia*. In the present case it seems to provide at any rate a partial explanation of the undoubted stimulating effect of *Colpidia* on ammonia production.

#### SUMMARY.

1. Two series of experiments were performed with a mixed culture of two species of soil bacteria with and without *Colpidia*, one series on a solution of peptone in soil extract, the other on a synthetic medium containing alanine.

2. An appreciable reduction in bacterial numbers was found on both media to occur in the *Colpidium* cultures as compared with the controls.

3. On the peptone medium the *Colpidium* cultures produced slightly more ammonia than the controls, in spite of their lower numbers of bacteria.

4. On the alanine medium the *Colpidium* cultures produced nearly the same amount of carbon dioxide and of ammonia as the controls.

5. In both series of experiments an inverse linear relation was found to exist between bacterial numbers and efficiency of ammonia production, and the same was found for carbon dioxide production in the second series.

6. The regression coefficients of efficiency on average bacterial numbers are significantly different in the *Colpidium* and control cultures for the second series.

7. It follows that the stimulating effect of the presence of *Colpidia* is not due solely to the reduction of bacterial numbers to an optimum value, and it is suggested that in the cultures in which *Colpidia* are present, the bacteria are kept in a state of youth for a longer period.

I should like to express my thanks to Mr Yates, of the Statistical Department, for valuable mathematical advice, and to Mr Ward Cutler, in whose department this work was carried out, for his constant help and encouragement.



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(Received April 16th, 1932.)

## REVIEW

*A Text-book of Practical Entomology.* By FRANK BALFOUR-BROWNE.

Pp. viii + 191 and 116 text-figs. London: Edward Arnold and Co., 1932. 18s. net.

The author states in the preface to this book that its object is "first, to let the student see as much as possible of the structure of the insect and of the modifications of those structures, but, at the same time to understand how the various parts work." The book is divided into three sections. Part I is an elementary course and is intended to acquaint the student with the general structure of the cockroach and how to recognise the different orders of insects in their adult and immature stages. Part II, which is styled an advanced course, deals with the general structure of *Dytiscus* both larva and imago. This account is followed by a series of short chapters of a comparative nature on the mouth-parts, wing-coupling apparatus, legs, respiratory system and aquatic adaptations. There are also some rather cursory remarks on embryology and histology. The concluding section of Part II appears to be designed to acquaint the student with the range of form found in different insect orders. Part III is described as a course on the principles of systematic entomology and is intended to teach the student those characters of importance in taxonomy. It starts with the special morphology of the grasshopper followed by that of representatives of the larger insect orders and their larvae. Wing venation is also considered in this section of the book. There are included two more or less detailed keys to the larvae of Coleoptera and Lepidoptera. The former is based on the rather antiquated table of Macgillivray and the latter upon the work of Fracker. It is not obvious, however, why these two keys are included since there are none to the adults of any order. Presumably they are intended to familiarise the student with the use of tabular diagnosis of this kind.

A special feature of the book are the numerous and clearly executed text-figures which are all original. These should prove an invaluable help to the student in interpreting the specimens before him. The author has not carried out his intention of teaching the student to understand "how the various parts work" with very great success. Wings and wing-sclerites, for example, are described and figured but there seems to be no account of how an insect is able to fly! Every teacher has his own ideas as to how a practical course should be planned, and the extent to which this book will be used will depend largely upon how far it suits the scheme of a particular teacher. It is well printed and free from misprints and there seem to be but few errors in the text.

A. D. IMMS.

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